Chemical Blood Studies.*

III. Comparative Studies on "Laked" and "Unlaked" Blood Filtrates of Sheep in Health and during "Heartwater" (Rickettsia ruminantium infection) and Bluetongue (Catarrhal fever).

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^{*&}quot;Chemical Blood Studies," I, III-V was accepted as Thesis for the D.V.Sc. degree by the University of Pretoria, December, 1932. For series of articles published up to date see "References."

I.—GENERAL INTRODUCTION.

The present article is the third of the above series (Graf 1933) and deals exclusively with chemical research work in connection with Heartwater and Bluetongue. It has for a long time been felt that the absence of biochemical data, particularly in respect of the blood constituents in diseases of domestic animals, has been a great drawback from the veterinarians and veterinary pathologists points of view. A clear conception of the pathology is not possible without a knowledge of the chemical processes involved. It is towards this aspect that these researches are ultimately directed. In order to avoid needless repetition, the objects, technique and the methods employed have been fully detailed in the first article (Graf 1933), page 269, in this Journal.

II.—A. HEARTWATER IN SHEEP.

It is beyond the scope of this paper to discuss in detail the distribution, symptomatology, pathology, etc., of this disease, but for those to whom this condition is unknown, a short note may be of interest. For a comprehensive review of our present state of knowledge in heartwater in all its aspects, I would refer to the most recent publications on this disease, viz.: by Alexander (1931), in which is included a full bibliography, and that by Jackson and Neitz (1932).

Heartwater may be defined as "a febrile septiceemic infectious disease of sheep, goats and cattle, caused by *Rickettsia ruminantium*, and transmitted by ticks of the genus *Amblyomma*, chiefly *A. hebraeum*." The disease receives its name from the common pathological lesion in sheep and goats, namely, a well marked hydropericardium, though this is not a constant nor the most pronounced finding in cattle. Animals which recover do not harbour the virus in the blood, which is infective during and for a short time after the reaction. Recovery from a reaction results in a solid, but not absolute immunity." (cit. Alexander.)

The disease can be readily produced in susceptible animals by the injection of 5-10 c.c. blood taken from a reactor during the actual time of reaction and for a short while thereafter. The incubation period is from 5-35 days, generally 7-14 days.

The symptoms of the disease cannot be regarded as pathognomonic. There is usually a sudden rise in temperature, which may go up to 198, followed by gradual loss of appetite and cessation of rumination. Associated with the hyperthermia there is usually dysphoea, rapid pulse, becoming weaker with the progress of the disease, cyanosis of the mucous membranes and nervous symptoms gradually supervene, the latter being present in the majority of cases. The nervous symptoms usually show in the form of unsteadiness of gait, unnatural position when standing or lying, when down galloping movements of legs, champing of the jaws with resultant frothing. The agonal period may extend up to 24 hours. The mortality varies considerably, being for Merino sheep about 60 per cent., in the present experiment 75 per cent.

At post-mortem few really characteristic lesions are found, but there is commonly cyanosis of the mucous membranes, hydroperitoneum, hydropericardium, subendocardial haemorrhages, cloudy swelling and fatty degeneration of the myocardium and oedema of the lungs, swelling, hyperaemia, fatty degeneration and bile stasis of the liver, hyperaemia and degeneration of the kidneys.

The histo-pathology of the disease has been studied by Steck (1928), who summarises his findings as follows: "Besides the presence of *Rickettsia ruminantium* (Cowdry) the characteristic changes in heartwater are leucostasis and perivascular cellulation. The former occurrs in all organs and the macrophage is the prominent cell, but lymphocytes and neutrophiles are also numerous. The latter is pronounced in the liver and kidneys and sometimes in the adrenal glands.

All the cells of the mesenchyma may take part in this reaction, but mainly those of the lymphocytic series, viz.: lymphocytes and plasma cells. It seems most likely that these alterations are due to a noxe which is spread diffusely by the blood stream. It must remain for further investigation to determine the nature of this noxe."

(a) SELECTION AND TREATMENT OF THE EXPERIMENTAL SHEEP.

In these researches sheep (Merinos) were specially selected from among a large herd purchased in heartwater-free areas of South Africa to ensure their being susceptible. Clinical examinations were made to ensure getting normal healthy sheep in good condition. Faecal examinations to determine the degree of helminthic infestation were also undertaken (through the courtesy of Dr. Mönnig). Only sheep with very light infections were utilised after being dosed with Wireworm Remedy, which dosing was furthermore carried out monthly.

The success of these precautions was amply demonstrated at the postmortem examinations where, with the exception of a few oesophagostome nodules, no adult worms could be found in any of the cases. Fourie (1931) reports on the morphological changes associated with haemonchosis, changes which probably also affect the chemical composition, and it was primarily to reduce or virtually eliminate such complications that the reduction of the degree of verminosis was undertaken. The sheep in all cases were placed on temperature for varying lengths of time before injection with heartwater virus, several different strains of it, including natural or veld strains being used. To the virulency of the strains employed, the short duration of the disease in most cases and the heavy mortality, 75 per cent., bear testimony. Only three of the twelve cases recovered.

(b) CHEMICAL METHODS AND TECHNIQUE.

For these see Chemical Blood Studies I (Graf 1933) (this Journal).

(c) Experimental Data.

In the preparation of this paper some difficulty was experienced as to what would be the most convenient method of presenting the large mass of data collected so as to bring out those features differing from the normal. The system finally decided upon was to briefly draw attention to the salient features in each case of heartwater emerging from the analytical data, treating each constituent separately and thereafter summarising the findings in such a way as to focus attention on the deviations from normal more or less common for all the eases studied.

As it is essential to know first the normal range of variation for each constituent before it can be known what is abnormal or pathological, the analytical figures obtained before the injection of virus have been collected and summarised. The numbers presented here are relatively few, but when discussing bluetongue, further normal data will be presented. During the last year detailed investigations on similar lines on normal healthy sheep have been carried out by Hamersma (1933) of this Division, and these will be published in the next number of this Journal (vide Chemical Blood Studies VI).

(1) Normal Range of Constituents of Blood Filtrates.

Sugar.—This was found to vary from 32.90 to 73 mgm. per cent. in the case of "laked" filtrate, the figures falling in the majority of cases between 45-55 mgm. per cent. In the "unlaked" filtrates the variations ranged from 26.20-68.50 mgm. per cent. with the majority of figures lying between 31-42 mgm. per cent. The amount of sugar determined was in all cases lower in the "unlaked" than in the "laked" to the extent of 15-40 per cent., with an average of approximately 25 per cent. In one case the difference was as little as 2 per cent., in another as much as 60 per cent.

Total Nitrogen.—The variations found ranged from $2 \cdot 2 \cdot 2 \cdot 9$ gm. per cent., both these figures being, however, only once determined, by far the greatest number falling within the $2 \cdot 3 - 2 \cdot 6$ gm. per cent. range—a remarkably constant and relatively narrow range. This is even more striking when the considerable variation in the haemoglobin content of the blood is considered, not only of the same animal at different bleedings, but also in the different animals when compared with each other.

Urea Nitrogen.—When summarising the "Urea N" content of the "laked" filtrates before injection of virus, figures varying from 3·42 mgm. N per cent. to 20·45 mgm. N per cent. (7·14-42·84 mgm. urea) are encountered. The following tables illustrate the distribution more clearly by showing the number of analyses falling into each particular group. Owing to the slight difference existing between the concentration of urea in the corpuscles and the plasma the "laked" and "unlaked" figures have been grouped together:—

From	3-4	mgm. N %	4	From 12-13 r	ngm. N % 0
	4-5	,,	7	13-14	,, 2
	5-6	,,	5	14-15	,, 2
	6-7	,,	4	15- 16	,, 0
	7 - 8	,,	3	16-17	,, 0
	8-9	,,	1	17-18	,, 3
	9-10	,,	1	18-19	,, 3
	10-11	,,	2	19-20	,, 0
	11 - 12	11	()	20-21	,, 2

From the above table it is evident that the majority of figures from "urea N" lie below 10 mgm. N per cent., viz., 25 out of 39, i.e., 64 per cent. It is noticeable that the first bloods done are mostly fairly high and, in my opinion, cannot be regarded as absolutely normal. Hamersma, in the work referred to earlier, dealing with hundreds of analyses, has found extraordinarily low values, less than 1.5 mgm. N per cent. The normal range lies from 2–10 mgm. N per cent., with the average at 4.7 mgm. N per cent. I would here also refer to the "normals" given in connexion with bluetongue (vide page 316), where the above findings are further substantiated.

Since the "urea N" in case of "laked" and "unlaked" filtrates is much the same, the same averages as given above apply. The "unlaked" filtrate, as a general rule, contains slightly less "urea N," the difference being, however, small—mostly less than 0.3 mgm. N per cent. The position with sheep is, as regards distribution of urea between plasma and cellular elements, thus similar to that recorded by Folin in human blood.

"Total Creatinine" Nitrogen.—This fraction represents the creatine N and the creatinine N, the creatine having been converted first into creatinine and the latter plus the preformed creatinine determined.

The variations in "laked" filtrates range from $2\cdot08-2\cdot66$ mgm. N per cent. ($5\cdot6-7\cdot2$ mgm. creatinine) and in one case only exceeds the maximum given here, viz., $3\cdot42$ mgm. N per cent. Approximately half the cases lie between $2\cdot08-2\cdot15$ mgm. N per cent. Although the variation is somewhat large in different sheep, figures for each individual are fairly constant, e.g., S. 32297 (Table 6), the amounts are $2\cdot23$, $2\cdot61$, $2\cdot23$, $2\cdot42$ mgm. N per cent. respectively, and in S. 29661 (Table 9) $2\cdot13$, $2\cdot13$ and $2\cdot31$ mgm. N per cent. respectively.

For "unlaked" filtrates the corresponding figures range from $1\cdot 4-2\cdot 15$ mgm. N per cent. $(4\cdot 0-5\cdot 8$ mgm. creatinine) being only exceeded in two cases with $2\cdot 23$ and $2\cdot 35$ mgm. N per cent. The variation is, therefore, greater than is encountered with in "laked" bloods, although the amount of "total, creatinine" N is lower.

Amino-Acid Nitrogen.—This varies in the case of "laked" blood filtrate from $5\cdot09-8\cdot00$ mgm. N per cent. (exceeded once only with $9\cdot72$) in the majority of cases falling into the $5\cdot30-6\cdot80$ mgm. N per cent. group. For "unlaked" filtrates the range lies from $3\cdot60-5\cdot96$ mgm. N per cent., with $4\cdot67-5\cdot64$ mgm. N per cent. being the largest group.

Uric Acid Nitrogen.—In the "laked" filtrate the normal variation is from 0·18–0·28 mgm. N per cent. (0·54–0·84 mgm. uric acid) with a narrower range of 0·18–0·23 mgm. N per cent. (0·54–0·69 mgm. uric acid). In the case of unlaked filtrates, determinations were always made, but the colour obtained was rather faint for accurate colorimetric readings when the dilutions were made up to 25 c.c. Subsequently a modification was introduced by diluting only to 15 c.c. when readings could be more accurately taken—a procedure that was followed in all later studies. The "normals" for uric acid will, therefore, be discussed under "Bluetongue" (vide page 316).

Non-Protein Nitrogen.—Great variations were noted here, the data ranging from $13\cdot76-30\cdot28$ mgm. N per cent. for "laked" and $10\cdot66-27\cdot70$ mgm. N per cent. for "unlaked" filtrates. The following two tables more clearly indicate this distribution and the groups in which most of the "normals" are concentrated.

(a) Table j	for "laked rates.	,,	(b) Table for filt	"unlaked" rates.
From 13-14		% 1	From 10-11	
14-15	,,	1	11-12	,, 1
15-16	,,	0	12-13	,, 2
16-17	,,	2	13-14	,, 3
17 - 18	22	0	14-15	,, 3
18-19	"	4	15-16	,, 3
19-20	. ,,	1	16-17	,, 1
20-21	,,	1	17-18	,, 0
21-22	,,	3	18-19	,, 1
22 - 23	,,	1	19-20	,, 1
23-27	2;	0	20-23	,, 0
27 - 28	,,	1	23 - 24	,, 1
28 - 29	22	1	24 - 25	,, 1
29-30	22	1	25 - 26	,, 0
30 - 31	22	2	26-27	,, 1
			27-28	,, 1

The largest number of analytical figures lie from 10–17 mgm. N per cent. The higher N.P.N. figures I am inclined to hold to be really outside the true normal range, but no reason can at the moment be offered in explanation. The sheep appeared clinically healthy. A larger mass of data is required before valid "normals" can be laid down. These, for South African conditions, will soon be published by Hamersma and in connexion with the researches into bluetongue, further figures are supplied.

Heartwater Data

Temperature Charts.—In order to indicate the type of temperature reaction, and more particularly to demonstrate at what periods of the reaction blood examinations have been made, charts have been incorporated. The periods at which blood has been drawn has been indicated on the curves by small circles. Temperatures were taken twice daily (once daily on Sundays) at 6.30 a.m. and 3.30–4 p.m., respectively. Only the actual reactions are recorded, the normal temperature records being omitted for the sake of economy of space, no useful purpose being served by the incorporation of several weeks of such normal records.

Tables of Data.—These are mostly self-explanatory, all constitutents being expressed in "mgm. per 100 c.c. of blood," except haemoglobin and total nitrogen, both of which are expressed as "grams per 100 c.c." The "coaguable nitrogen" has been obtained by calculation (Total N—N.P.N.). The "Rest nitrogen" represents the nitrogen fraction unaccounted for in any specific form after the "urea N" "Total creatinine N," "uric acid N" and "Amino acid N" had been subtracted from the "Non-protein N" figure. The urea (46.66 per cent. N), total creatinine (37 per cent. N) and uric acid (33.33 per cent. N) have for the sake of convenience been expressed both as such and as "Nitrogen." In the column "plasma" the symbols "n.u." (nothing unusual) refer to the physical appearance of the plasma, more particularly to its colour. In anaplasmosis, redwater and horsesickness, icteric plasmas were quite frequently encountered. The "plasma" column has been omitted in the case of heartwater, since the plasma at no time showed any haemolytic or icteric discoloration.

In the column "Temperature Reaction" the following symbols have been adopted:—

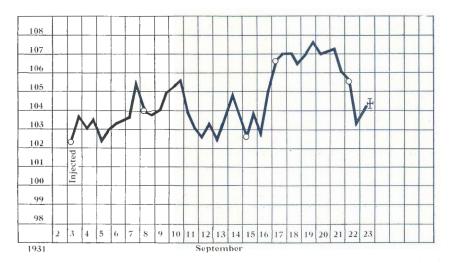
- N (Normal)—meaning that no abnormal temperature reaction is going on at the time of bleeding.
- P.I.N. (Post infectionem, normal)—indicating that the animal has been injected with virus, but that as yet no temperature reaction has set in.
- R (Reaction)—indicating that blood was withdrawn during the course of a reaction.
- "Time of bleeding"—where no symbol is given it means that the blood was drawn between 8.30 a.m. and 9.30 a.m. In other cases the time of bleeding is inserted.

The above system has been applied throughout these publications.

Heartwater (Rickettsia ruminantium infection).

CASE T.

S. 27088: Killed in extremis, 23/9/31.



History.—Sheep 27088, hamel, six-tooth, carrying $1\frac{1}{2}$ ins. wool, in good condition, weighing 109 fb. on 15/9/31. Passed through bluetongue in July, 1931. Was placed on temperature 2/9/31, and was injected intrajugularly on 3/9/31 with 10 c.c. mixed blood from heartwater sheep S. 32189 and S 31874. There was a temperature reaction from 4th to 8th day p.i. but there was no reflection of this slight reaction in the composition of the blood as examined on 8/9/31. The heartwater reaction began on 13th day p.i., the temperature rising within 36 hours to 107° , remaining at this level for four days and then dropping by crisis to $103 \cdot 2^{\circ}$, the animal being killed in extremis on 20th day p.i. At the height of the reaction blood was injected into S. 31045 and S. 31761 (vide).

At the post-mortem examination (P.M. No. 10586, 23/9/31) the following pathological changes were found: slight hydrothorax and hydropericard, subendocardial haemorrhages, oedema of the lungs, tumor splenis, slight catarrhal enteritis, Preisz-nocard abscess in lung, fat necrosis, few oesophagostome nodules in caudal part of small intestines.

TABLE I.

S. 27088. Date Time	31/8/31.	3/9/31.	8/9/31.	15/9/31.	17/9/31.	22/9/31.
$\overline{Temp. R}$	N	N	P.I.N.	R	R	R
Hb. gm. %	13.79	13 · 12	13.31	12.13	11.78	11.67
Sugar mgm. % L	56·80 46·30	$39.53 \\ 34.01$	51.00	77·70 60·70	93·10 40 ?	50·00 28·6
T.N. gm. %	2.940	2.774	2.674	2.506	2 · 294	2.611
<i>N.P.N.</i> mgm. % L U	16·08 12·62	14·60 11·75	14.75	15·15 11·70	18·07 12·50	$26.55 \\ 20.54$
Coag. N. L gm. N % U	$2 \cdot 924 \\ 2 \cdot 927$	$2 \cdot 759 \\ 2 \cdot 761$	2 · 659	$2 \cdot 491 \\ 2 \cdot 494$	$2 \cdot 276 \\ 2 \cdot 282$	2.584 2.590
Urea mgm. %L	4·20 8·82 3·80 7·98	$4 \cdot 23$ $8 \cdot 82$ $4 \cdot 18$ $8 \cdot 82$	5·13 10·71 —			
Total creatinine L mgm. N %	=			$2 \cdot 66$ $7 \cdot 20$ $1 \cdot 78$ $4 \cdot 80$	$ \begin{array}{r} 2 \cdot 23 \\ 6 \cdot 00 \\ 2 \cdot 23 \\ 6 \cdot 00 \end{array} $	$ \begin{array}{r} 1 \cdot 90 \\ 5 \cdot 12 \\ 2 \cdot 08 \\ 5 \cdot 60 \end{array} $
Uric Acid L mgm. % U	0·19 0·57 TL TL	$0.23 \\ 0.70 \\ \text{TL} \\ \text{TL}$	0·23 0·68 TL TL	0·21 0·62 TL TL	$\begin{array}{c} 0 \cdot 21 \\ 0 \cdot 62 \\ \text{TL} \\ \text{TL} \end{array}$	0·17 0·51 TL TL
Amino-acid L mgm. % U	8 4·68	6·28 4·88	5.13	$5 \cdot 76 \\ 4 \cdot 38$	$6 \cdot 19 \\ 4 \cdot 38$	5·56 4·81
R.N. mgm. N % L	4·68* 3·14‡	3·86* 2·69‡	5 · 26*	7 · 52† 6 · 54§	10·34† 6·89§	19·92† 14·65§

^{*} Includes "Total creatinine N."

Salient Features Emerging from Analytical Data.

Hb.—A definite and steady drop from 13.79 gm. per cent. to 11.67 gm. per cent.

Sugar.—"Laked" filtrate shows a marked rise (51-93 mgm. N per cent.) corresponding to set in of the temperature reaction, followed by a drop to initial level on day preceding death. In the "unlaked" the reverse occurs.

T.N.—Shows a decrease succeeded by a rise towards end.

N.P.N.—Both "laked" and "unlaked" filtrates show a steady rise from 15·15-26·55 mgm. N per cent. and 11·70-20·54 mgm. N per cent. respectively.

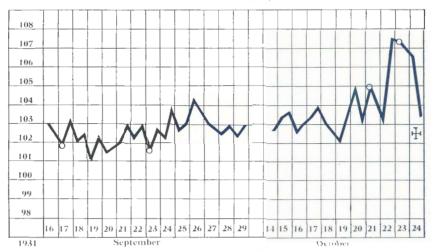
T.C.N.—Data incomplete, but in the case of "laked" filtrate there is a definite decrease (2.66-1.90 mgm. N per cent.). In the "unlaked" filtrate an initial rise is succeeded by a decrease on the day prior to death. The variations are within the normal range and may be of no special significance.

[&]quot; "Urea N."

[&]quot;Total creatinine and uric acid N."
"Urea N" and "Uric acid N."

- U.A.N.—The variations are small and within the experimental error and normal variation, but there seems a tendency towards a decrease as the disease progresses.
- A.A.N.—The variations are within the normal range and no clear increase or decrease can be detected.

Case II. S. 26689: Died 24/10/31.



History, -Sheep 26689, hamel, full mouth, carrying 13 ins. wool, good condition, weighing 84 fb. on 15/9/31 and 87 fb. on 6/10/31. Passed through bluetongue in July, 1931. Was placed in heartwater experiment on 2/9/31 and injected on 3/9/31 intrajugularly with 10 c.c. blood from S. 32179 and S. 31874. After 17 days p.i. there was a slight temperature reaction, the maximum of 104.4° being reached on the 22nd day p.i., dropping to below 103° 48 hours later. This could not be regarded as a typical temperature reaction for heartwater in sheep or at the most as only a very mild reaction. It is, however, of interest to note that a blood examination undertaken on 23/9/31 just prior to the rise in temperature shows a marked rise in the "Urea N" content, viz.: 20.60 mgm. per cent. and 19.40 mgm. per cent. respectively for laked and unlaked filtrates. If this is compared with subsequent analyses taken during a later reaction, the suggestion is permissive, that the reaction is possibly a mild heartwater reaction. On the other hand, it should be noted that, if this reaction was due to heartwater, it conferred no immunity since exactly one month later (23/10/31) this animal succumbed to heartwater.

On 12/10/31 this sheep was again injected intrajugularly with 10 c.c. blood from S. 31109 (vide). On the 6th day p.i. the temperature reaction set in, the peak of 107·6° being reached four days later, the temperature falling during the next 48 hours by crisis to 104°, the animal dying on the 12th day p.i.

On post-mortem examination (P.M. No. 10548 of 25/10/31) there was found to be present marked hydrothorax, severe hydropericard, cyanosis of mucous membranes, oedema and hyperaemia of the lungs, subepicardial and subendocardial haemorrhages and slight catarrhal gastro-enteritis. The post-mortem findings, therefore, supported the diagnosis of heartwater.

TABLE II.

ctions	9 (0 / 9 1							_
	о/а/от.	8/9/31.	15/9/31.	17/9/31.	23/9/31.	13/10/31.	21/10/31.	23/10/31.
C C C	N	P.L.N.	P.I.N.	a	M.	P.I.N.	R	83
T C C	9.50	10.27	11.39	11.47	11.53	10.76	10.24	10.99
	51.81 38.46	58.92	60 · 45 37 · 00	35.00 30.00	46.70 37.40	53.19 35.80	50 · 00 48 · 54	79.00 64.90
Total N	2.401	2.373	2.429	2.429	2.590	2.527	2.569	2.590
N.P.N. mgm. % L 17.80	13 · 76 10 · 60	21.13	21.90	19 -36	35·30 32·60	16.48 12.50	23.62 20.68	35 · 30 28 · 56
Coag, N. gm. N % L 2.355	2 · 387 2 · 390	2 · 352	2 · 407 2 · 414	2.410 2.414	2 · 555 2 · 557	2.510 2.515	2.545 2.548	2.555 2.561
Urea mgm. N % L 4.70 9.87 U 3.50	3.42 7.40 3.40 7.14				21.60 45.36 19.40 40.74	8·61 8·61 3·70 7·56	11.46 24.15 11.42 23.94	21.68 45.57 21.40 44.94
Total Creatinine mgm. N % L		2.54	2 · 54 6 · 86 1 · 98 5 · 32	2.87 7.74 2.66 7.20	2 · 34 6 · 36 6 · 36 6 · 36	1.91 5.14 1.87 4.88	2.06 5.54 1.95 5.24	2.31 6.26 1.91 5.14
Uric acid mgm. N % L	0.27 0.81 TL TL	0.28 0.84 TL TL	0.32 0.96 TL TL	0.23 0.78 TL TL	0.21 0.63 TL TL	0.21 0.62 TL TL	0.66 2.00 0.19 0.58	0 · 28 0 · 83 TL TL
Amino-acid mgm. N % I, 7.30 U 5.10	6.80	6.22	7.18	7.45	7 · 48 5 · 87	7.29	6-67	5·18 3·50
R.N. mgm. N % U 6.80*	3.27† 2.50*	13.09‡	12.86‡ 8.38\$	9.81‡ 8.00§	4 ·48 5 ·68	2.73	2.77	5.85

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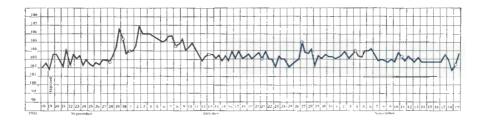
Salient Features Emerging from Analytical Data.

Hb.—Nothing unusual.

- Sugar.—During the initial slight reaction (nature uncertain) the level is very, low gradually rising thereafter in both "laked" and "unlaked" filtrates, reaching a level of 79 and 65 mgm. respectively on day before death.
- T.N.—A tendency towards an increase exists, but the level remains within the normal limits. The increase is too small to permit of attaching any particular significance to it.
- N.P.N.—Both filtrates show an increased nitrogen content during both the initial mild hyperthermic reaction and the second severe reaction (heartwater), with a return to normal in the interval between the two reactions.
- U.N.—Similar to the N.P.N. curve, but much more marked, e.g., a rise from ± 4 mgm. N per cent. to 21 60 mgm. N per cent., with a drop to the initial level and a further marked increase during the second reaction.
- T.C.N.—No very definite changes, but with a tendency towards an increase with the rise in temperature.
- U.A.N.—Nothing definite, although a high U.A.N. content was encountered three days before death.
- A.A.N.—Nothing usual.

Case III.

S. 31045: Recovered.



History.—Sheep 31045, two-tooth hamel, carrying $2\frac{1}{2}$ ins. wool, fair condition, weighing 77 fb. on 15/9/31 and 80 fb. on 6/10/31. Passed through bluetongue in May, 1931. It was placed on temperature on 2/9/31 and was injected with 10 c.c. blood from S. 27088 (vide) on 19/10/31. The temperature reaction set in on the 10th day, reaching 106.8° on the 11th day, decreasing by lysis during the next twelve days to normal, i.e., the 22nd day p.i. The temperature remained normal, and on 1/12/31 an immunity test was performed, no reaction resulting.

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TABLE III.

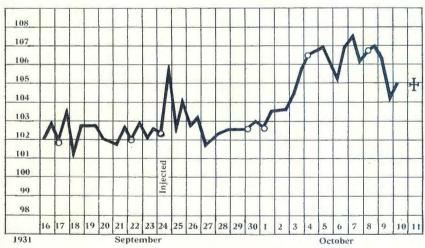
S. 31045.	-											
Date	22/9/31.	28/9/31.	30/9/31.	1/10/31.	8/10/31.	13/10/31.	13/10/31. 21/10/31. 27/10/31.	27/10/31.	4/11/31.	11/11/31.	11/11/31. 19/11/31.	1/12/31.
Temperature Reactions	P.I.N.	P.I.N.	R	R	R	N	Z	23	S.R.	N	N	N
Haemoglobin gm. %	10.56	9.94	10 · 14	10.14	9.32	8 - 49	9.81	9.81	8 - 49	8 - 49	10.14	10.99
Sugar mgm. % L		43.48	73 · 56 58 · 12	55 · 55 44 · 44	29.70 25.00	52 · 63 45 · 45	47 ·62 35 ·71	60.00	36.00 26.22	50.25	42 · 55 35 · 71	42.37 38.30
T.N. gm. N %	2.500	2.392	2.376	2.416	2.192	2.262	2.080	2.318	2.059	2.080	2.318	2.584
N.P.N. mgm. % L	20.70	18.07	19 · 73 15 · 39	26.20 21.90	29.36 24.06	19.23 15.85	18.18 14.28	28.82 21.82	30 ·30 25 ·20	20 · 42 15 · 00	27 · 90 21 · 43	18 · 75 13 · 50
Coag. N. gm. N % L	2.479	2.374	2.356	2.390	2.163	2.243	2.062	2.289	$\frac{2.029}{2.034}$	2.060	2.290	2.565
Urea mgm. N % L	7.32 15.33 7.00 14.70	5.73 11.97 5.55 11.76	7 · 03 14 · 70 7 · 13 14 · 91	10 · 63 22 · 26 10 · 83 22 · 68	17 · 61 36 · 96 17 · 25 36 · 33	7.82 16.38 7.76 16.38	7.26 15.33 6.17 13.02	13 · 14 27 · 51 13 · 73 28 · 77	18.80 39.48 18.70 39.27	7.00 14.70 6.14 12.81	11.79 24.78 11.25 23.73	4.92 10.29 5.13 10.71
Total Creatinine mgm. N % L	2 · 08 2 · 00 5 · 54 5 · 54	2.31 6.36 2.01 5.40	1.91 5.14 1.78 4.80	2.31 6.26 1.98 5.32	2·13 5·76 1·82 4·90	1.98 5.32 1.82 4.90	1.91 5.14 1.82 4.90	1.78 4.80 1.82 4.90	2.17 5.86 1.78 4.80	2.42 6.54 2.13 5.76	2 · 42 6 · 54 2 · 23 6 · 00	2.31 6.26 2.17 5.86
Uric acid mgm. N % L	and the second s		1111		1 [] [0.26 0.79 TL TL	0.20 0.61 0.13 0.40	0 · 20 0 · 60 TL TL	0.18 0.54 TL TL	0.17 0.51 TL TL	0.15 0.44 TL TL	0.24 0.73 0.14 0.43
Amino-acid mgm. N % L	6.33	6.54	6.54	7.91	5.96	7.87	6.60	7.57	5.83	6.09	7.00	6.09
R.N. mgm. N % U	5.07*	3.45* 2.14*	4.25*	5.35*	3.66*	1.30 0.46*	$\begin{array}{c} 2.20 \\ 1.07 \end{array}$	6.13 1.89*	3.32 0.81*	4.72	6.54 1.59*	5·19 0·82

* Includes " Uric acid N."

Salient Features Emerging from Analytical Data.

- Hb.—Shows tendency towards a drop in the Hb level during the hyperrhexic state.
- Sugar.—Rather variable, the highest content being encountered during the hyperrhexias with 73.56 and 60 mgm. per cent. respectively, following by a decrease beyond the average level.
- T.N.—Shows a gradual decrease, the lowest points being reached after the reaction has already returned to normal.
- N.P.N.—A definite increase coinciding with the two temperature reactions.
- U.N.—Both filtrates show a definite increase coinciding with the increase in body temperature from an initial level of \pm 7 mgm. N per cent. to 18 mgm. N per cent.
- T.C.N.—Variations cannot be definitely associated with the progress of the condition. With the second more severe hyperrhexia there is diminution of the "creatinine N" content, followed by a rise, but the differences encountered do not fall beyond the normal limits and may be coincidental.
- U.A.N.—With the second reaction is associated a definite drop from 0·25 mgm. N per cent. to 0·15 mgm. N per cent. This decline is not directly dependent on body temperature, as it proceeds after the temperature has returned to normal.
- A.A.N.—A decrease coinciding with the temperature reaction.

Case IV. S. 31028: Died 10/10/31.



History.—Sheep 31028, four-tooth hamel, carrying $1\frac{1}{2}$ ins. wool 14/9/31, in fair condition, weighing 66 fb. on 15/9/31 and 67 fb. on 5/10/31. It passed through bluetongue reaction in April, 1931, and was placed in a bloedpens experiment during May, 1931, being drafted into the heartwater experiment on 2/9/31. It was injected with 10 c.c. blood mixture from S. 29651 and S. 28462 during the height of a heartwater reaction in these animals on 24/9/31. A severe heartwater reaction set in on the 9th day p.i., the

TABLE IV.

			TABLE JV.	JV.				
S. 31028. Date Time.	::	17/9/31.	22/9/31.	24/9/31.	30/9/31.	1/10/31.	4/10/31.	8/10/31.
Temperature Reactions		N	N	N	P.I.N.	P.I.N.	R	R
Haemoglobin gm. %	1:	11 -59	10.56	9 - 73	9.12	9.05	9.10	9.32
Sugar mgm. %	T C	51·13 38·84	38 · 20 26 · 20	44 · 60 31 · 20	57 · 47 57 · 14	57 · 02 41 · 60	62 · 43 48 · 00	50 · 00 39 · 20
T.N. gm. %	:	2.410	2 · 442	2.330	2.290	2 .332	2.310	2.339
N.P.N. mgm. %	45	22 · 90 15 · 70	22·10 14·92	35·30 29·30	26 · 09 22 · 90	20.00 15.36	48·89 43·36	63 ·84 53 ·58
Coay. N gm. N %	35	2.387	2 · 419 2 · 427	2.295 2.301	2.254 2.257	2.312	2 · 263 2 · 269	2 · 275 2 · 285
Urea mgm. N % U % N % N % U %.	Jp Jb			.	13.71 28.77 13.00 27.30	7.73 16.17 7.55 15.96	32.06 67.41 31.83 66.78	49.18 88.62 37.37 78.54
7.C. mgm. N %. TC %. " N %. " TC %.	D C	2 · 40 6 · 48 1 · 98 5 · 32	3 · 42 6 · 54 2 · 23 6 · 00	2 · 66 7 · 20 1 · 67 4 · 50	2.13 5.76 5.06 5.54	2 · 06 5 · 54 1 · 75 4 · 70	1.98 5.32 1.67 4.50	2.54 6.86 2.17 5.84
Uric Acid mgm. N % UA % N % UA % UA %	n n	0·18 0·53 TL TL	TE	0.21 0.62 TL TL	11.		TI.	
Amino Acid mgm. N %	JP	5.83	6.39	5.74	7.37	6.54	6 .39	5-56
R.Y. mgm. N %	111	15.49* 9.59†	13 -29+	20 ·69* 22 ·63†	2 2 88 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	3 · 67‡	8 · 46‡ 4 · 66‡	13.56‡
* [ncludes " Urea N."	 	acludes "Urc	a N " and "	† Includes " Urea N " and " Uric acid N."	i	‡ Includes " Uric acid N."	I N."	

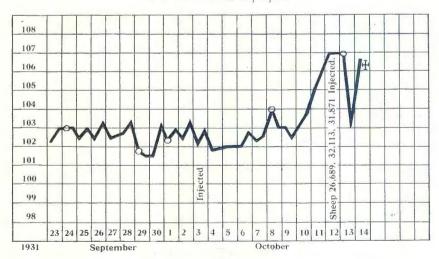
temperature maximum being $107 \cdot 6^{\circ}$ dropping by crisis on 15th day p.i., when the animal died. The post-mortem findings (P.M. No. 10624 of 11/10/31) supported the diagnosis of heartwater, the chief pathological findings being cyanosis of the mucous membranes, slight hydrothorax, hydropericard, hydroperitoneum, subendocardial and subepicardial haemorrhages and oedema of the lungs, fatty degeneration of the liver, catarrhal haemorrhagic gastritis, few oesophagostome nodules in the latter portion of the small intestines.

Salient Features emerging from Analytical Data.

- Hb.—A drop in this is noted, but this would not appear to be associated with the pathological condition since the decrease had already set in before even the virus was injected. On the contrary, there are indications of a slight increase during the hyperrhexic state.
- Sugar.—Shows an increase level during reaction succeeded by a fall shortly before death.
- T.N.—Slight decrease, possibly related to Hb. content.
- N.P.N.—Show a definite and marked rise in both filtrates, the N.P.N. and up to 63.84 mgm. N per cent. and 53.58 mgm. N per cent. respectively; the U.N. from about 7 mgm. N per cent. to 32 mgm. N per cent.
- T.C.N.—This animal shows initially a high level for T.C.N., but during the reaction there is a distinct if slight drop, with again a higher T.C.N. shortly before death. This applies to both filtrates.
- R.N.—Although the U.A.N. is included there is a marked increase towards the end of reaction.

CASE V.

S. 31109: Died 14/10/31.



History.—Sheep 31109, four tooth hamel, carrying 2 ins. wool, good condition, weighing 87 fb. on 15/9/31 and 96 fb. on 6/10/31. Passed through bluetongue in April, 1931, and placed in heartwater experiment on 2/9/31. Was injected on 3/10/31 with 5 c.c. blood intrajugularly from heartwater sheep S. 31761 (vide) and S. 31426. On the 6th day p.i. the temperature reaction set in, the temperature steadily rising over three days to 107°, dropping by crisis to 103° within 24 hours, the temperature rising by next morning again to 106·8°, the animal dying shortly after on the 11th day p.i. At the height of the reaction blood was injected from this sheep into S. 26689 and S. 32113 and S. 31871 (vide).

At the post-mortem examination (P.M. No. 10630 of 15/10/31) the pathological changes consisted of hydropericard, hydroperitoneum, hydrothorax, hyperaemia and oedema of the lungs, and slight tumor splenis.

TABLE V.

S. 31109.					
Date	24/9/31.	29/9/31.	1/10/31.	8/10/31.	13/10/31
Temp. React	N	N	N	P.I.N.	R
Hb. gm. %	12.01	12.42	11.80	14.95	9.73
Sugar mgm. %L	$\frac{32 \cdot 90}{26 \cdot 30}$	48·78 31·16		$51 \cdot 00 \\ 34 \cdot 10$	53·19 36·60
T.N. gm. %	2.680	2.710	2.521	2.934	2.388
N.P.N. mgm. %L U	34·50 31·60	36·60 34·60	28·58 22·00	$36 \cdot 36 \\ 29 \cdot 00$	27·78 25·27
Coag. N gm. N %L		$2.673 \\ 2.675$	$2 \cdot 492 \\ 2 \cdot 499$	$2.898 \\ 2.905$	$2 \cdot 359 \\ 2 \cdot 363$
Urea mgm. N %L	$\begin{array}{c} 20 \cdot 45 \\ 43 \cdot 05 \\ 20 \cdot 02 \\ 42 \cdot 00 \end{array}$	18:46 38:85 18:60 39:06		20·86 43·89 20·86 43·89	17·34 36·33 17·06 35·91
Total Creatinine L mgm. N % U	2·10 5·68 2·15 5·80	$2 \cdot 13$ $5 \cdot 76$ $1 \cdot 91$ $5 \cdot 14$	Ξ	$2 \cdot 31$ $6 \cdot 26$ $1 \cdot 98$ $5 \cdot 32$	$2 \cdot 06$ $5 \cdot 54$ $1 \cdot 98$ $5 \cdot 32$
Uric Acid mgm. N %L	0·24 0·72 TL TL	0·20 0·59 TL TL	0·17 0·50 TL TL	0·21 0·63 TL TL	0·30 0·89 TL TL
Amino Acid mgm. N %L	7·00 5·58	$7 \cdot 33$ $4 \cdot 62$	5.88	7·07 4·75	6·97 4·95
R.N. mgm. N %L	4·71 4·29*	8·48 9·47*		5·91 1·48*	2·11 1·28*

^{*} Includes "Uric acid N."

Salient Features Emerging from Analytical Data.

Hb.—Very irregular, lowest content on day prior to death.

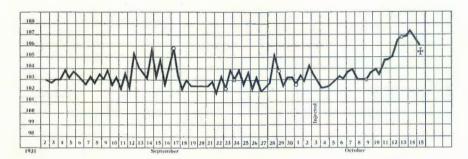
Sugar.—A tendency towards an increase.

N.P.N.—It will be noted that both these N fractions of the blood remained and consistently high over the entire period over which this animal U.N. was examined. No explanation can at present be offered since neither a clinical examination nor the port-mortem findings suggest any specific cause.

CASE VI.

S. 32297: Died 15/10/31.

Temperature Chart VI.



History.—Sheep 32297, two-tooth hamel, carrying 1 in. wool, condition fair, weighing 55 fb. 15/9/31 and 70 fb. 6/10/31. Suffering from ophthalmia of right eye on 14/9/31. Passed through bluetongue in May, 1931. Placed into heartwater experiment on 2/9/31 and injected with 5 c.c. blood intrajugularly from heartwater sheep 31761 (vide) and S. 31426 on 3/10/31. From the 8th day p.i. the temperature began to rise till the 11th day, reaching $107 \cdot 2^{\circ}$, dropping during the next day to 106° , the animal dying in the late afternoon, i.e., on the 12th day p.i.

On post-mortem examination (P.M. No. 10632 of 16/10/31) there was found to be present hydropericardium, slight oedema and hyperaemia of the lungs, slight tumor splenis and impaction of the oesophagus. No worms were found in the stomach and intestines. The condition of the carcass was good. The post-mortem findings, therefore, support the diagnosis of heartwater.

TABLE VI.

S. 32297.						
Date	17/9/31.	23/9/31.	29/9/31.	1/10/31.	9/10/31.	13/10/31
Temp. R	R	R	R	N	P.I.N.	R
Hb. gm. %	11.59	10.56	11.59	11.59	11.96	8.12
Sugar mgm. % L	41·66 31·00	39·30 34·40	38·46 33·78	_	57·14 42·01	96·16 44·44
T.N. gm. %	2.575	2.571	2.613	2 · 559	2.500	2.112
N.P.N. mgm. % L U	21·43 19·60	28·57 26·10	23 · 44	21·90 13·67	$25 \cdot 00$ $22 \cdot 31$	22·64 20·98
Coag. N. L gm. N % U	$2.554 \\ 2.555$	$2 \cdot 542 \\ 2 \cdot 545$	2.590	$2 \cdot 537 \\ 2 \cdot 545$	$2 \cdot 475 \\ 2 \cdot 478$	$2.099 \\ 2.101$
Urea mgm. N % L	$10 \cdot 94$ $22 \cdot 89$ $10 \cdot 69$ $22 \cdot 47$	$17 \cdot 35$ $36 \cdot 54$ $17 \cdot 02$ $35 \cdot 70$	$ \begin{array}{r} 8 \cdot 09 \\ 17 \cdot 01 \\ 9 \cdot 71 \\ 20 \cdot 37 \end{array} $	$6 \cdot 30$ $13 \cdot 23$ $4 \cdot 40$ $9 \cdot 24$	$14 \cdot 51$ $30 \cdot 45$ $14 \cdot 21$ $29 \cdot 82$	$ \begin{array}{r} 12 \cdot 50 \\ 26 \cdot 25 \\ 12 \cdot 94 \\ 27 \cdot 09 \end{array} $
Total Creatinine L mgm. No. % U	$2 \cdot 23$ $6 \cdot 00$ $1 \cdot 98$ $5 \cdot 32$	2·61 7·06 2·35 6·36	$2 \cdot 23$ $6 \cdot 00$ $2 \cdot 06$ $5 \cdot 54$	2·42 6·54 1·75 4·70	$2 \cdot 04$ $5 \cdot 50$ $2 \cdot 01$ $5 \cdot 40$	$2 \cdot 23$ $6 \cdot 00$ $1 \cdot 84$ $4 \cdot 96$
Uric Acid L mgm, N % U	0·27 0·80 TL TL	0·23 0·68 TL TL	0·21 0·62 TL TL	0·18 0·53 TL TL	0·18 0·53 TL TL	0·21 0·64 TL TL
Amino Acid L mgm. N % U	$5.64 \\ 4.97$	5·09 4·67	6·31 4·49	5·09 3·60	6·01 4·93	$5 \cdot 15 \\ 4 \cdot 20$
R.N. mgm. N % L	2·35 1·96*	3·29 2·06*	6.60	8·81 0·88*	2·26 1·16*	2·55 2·00*

^{*} Includes "Uric acid N."

Salient Features of Analytical Data.

Hb.—A distinct drop just prior to death.

Sugar.—Shows a marked rise to 96·16 mgm. per cent. and 44·44 mgm. per cent. in "laked" and "unlaked" filtrates respectively. In the "laked" the relative rise is more evident, and it is of interest to note that the increase in the case of the "unlaked" is not parallel to the former, the blood sugar being less in the latter by over a 100 per cent. This aspect will be discussed later under "General Discussion."

T.N.—A decrease is noted towards exitus lethalis.

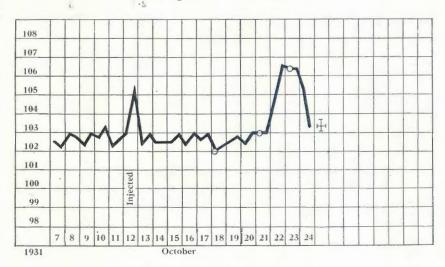
N.P.N.—No definite variations, the level throughout being on the high side. For this condition a temperature reaction of unknown etiology during September is apparently responsible, it being reflected by the slightly increased N.P.N. during this period.

- U.N.—The initial high U.N. is associated with the reaction referred to above, the normal level being again reached prior to the injection with virus, succeeded by a slight rise during the heartwater reaction.
- U.A.N.—During the first temperature reaction this fraction is just above the upper limit of normal, gradually diminishing.

CASE VII.

S. 31871: Died 24/10/31.

Temperature Chart VII.



History.—Sheep 31871, hamel, four-tooth, carrying 1 in. wool on 14/9/31, fair condition, weighing 54 fb. on 15/9/31 and 59·5 fb. on 6/10/31. Passed through bluetongue reaction in April, 1931. Was placed into the heartwater experiment on 2/9/31 and injected intrajugularly on 12/10/31 with 10 c.c. blood from heartwater sheep 31109 (vide). On the 9th day p.i. the temperature reaction set in, rising within 24 hours to 106·8° and dropping during the next 48 hours to 103·2°, the animal dying on the 13th day p.i. On the day prior to death 10 c.c. blood was injected intrajugularly into sheep 29661 and sheep 31011 (vide). On post-mortem examination (P.M. No. 10649 of 26/10/31) the following pathological changes were noted: hydropericard, hydrothorax, cyanosis of mucous membranes, degeneration of myocard, subendocardial and subepicardial haemorrhages, slight tumor splenis and a slight oedema of the lungs. The carcass was in fair condition. The post-mortem findings, therefore, support the diagnosis of heartwater.

TABLE VII.

S. 31871.					
Date Time	18/9/31.	28/9/31.	1/10/31.	21/10/31.	23/10/31
Temp. R	N	N	N	R	R
Hb. gm. %	9.52	8.49	9.52	9.32	9.40
Sugar mgm. %L	73·00 68·50	52·63 45·45	50·00 30·00	60·00 48·00	68·50 70·43
T.N. gm. %	2.362	2.180	2.340	2 · 213	2.024
N.P.N. mgm. %L U	19·67 14·51	30·90 27·70	16·82 13·49	22·23 21·63	40·54 35·30
Coag. N gm. N %L	$2 \cdot 342 \\ 2 \cdot 347$	$2 \cdot 149 \\ 2 \cdot 152$	$2 \cdot 323 \\ 2 \cdot 327$	$2 \cdot 191 \\ 2 \cdot 191$	1·983 1·991
Urea mgm. N % U		$18 \cdot 19$ $38 \cdot 22$ $17 \cdot 84$ $37 \cdot 38$	4·29 9·03 4·47 9·45	12·20 25·41 11·40 23·94	$24 \cdot 25$ $51 \cdot 03$ $23 \cdot 92$ $50 \cdot 19$
Total Creatinine mgm. N % L	$2 \cdot 15$ $5 \cdot 80$ $1 \cdot 67$ $4 \cdot 50$	2·66 7·20 2·10 5·68	$2 \cdot 31$ $3 \cdot 26$ $1 \cdot 32$ $4 \cdot 90$	$2 \cdot 23$ $6 \cdot 00$ $1 \cdot 98$ $5 \cdot 32$	$2 \cdot 23$ $6 \cdot 00$ $1 \cdot 98$ $5 \cdot 32$
Uric Acid mgm. N %L	0·22 0·66 TL TL	0·22 0·66 TL TL	0·22 0·66 TL TL	0.37 1.12 0.21 0.64	0.23 0.69 0.15 0.45
Amino acid mgm. N %L	$6.03 \\ 4.47$	7·00 4·95	$6 \cdot 28 \\ 4 \cdot 97$	6·83 5·96	5·18 4·67
R.N. mgm. N %L		3·05 1·81*	3·72 2·23*	$0.59 \\ 2.08$	7·50 4·73

^{*} Includes "Uric Acid N."

Salient Features of Analytical Data.

Sugar.—Shows an increase with rise in temperature.

T.N.—On the 28th September a drop occurs, associated with a low N.P.N. Hb content and a relatively increased N.P.N. and U.N. not and associated with any hyperthermia. During the heartwater U.N. reaction the T.N. and N.P.N. again diminishes, in this case unassociated with a decline in the Hb. level.

U.A.N.—With the onset of the reaction this is increased but rapidly drops to the normal level.

R.N.—This fraction is relatively high shortly before death.

CASE VIII. S. 32113: Recovered. Temperature Chart VIII.



History.—Sheep 32113, four-tooth hamel, carrying 1 in. wool on 14/9/31, in good condition, weighing 77 fb_on 14/9/31 and 80 fb. on 6/10/31. Passed through bluetongue in June, 1931. This animal was placed on temperature on 2/9/31. It was injected on 12/10/31 with 10 c.c. blood from S. 31109, which was suffering from heartwater. Six days later the temperature rose, reaching 106° on the 8th day, dropping to 103° on the 10th day, rising again on the 9th day to 107°, remaining at between 107° and 108° for six days and then decreasing by lysis to reach 103° in twelve days (27th day p.i.). On 1/12/31 it was again injected with 5 c.c. virulent heartwater blood, but no reaction occurred.

Salient Features of Analytical Data.

Temperature Reaction.—The hyperthermia encountered here is atypical for heartwater, although no doubt as to its origin exists. It is a severe long drawn out reaction followed by a milder, though distinct hyperthermia, during which the blood changes are similar to those encountered during the main reaction only to a somewhat lesser degree. It ended in ultimate recovery, but from a clinical point of view the issue was doubtful for several days, the animal being down for 72 hours. In my opinion the long drawn out temperature reaction is an expression of the strong resistance put up against the infection. This case, therefore, represents a more complete and accurate picture as to the changes involved in the composition of the blood during heartwater than is the case of a reaction ending abruptly in death.

Hb.—A well-marked decrease in the Hb. content (from 9·12-7·68 gm. per cent.) is noted, coinciding with the main reaction.

Sugar.—Shows an increase during the main reaction, preceded by a decrease at its onset.

T.N.—Runs parallel with the Hb. content, the decrease in T.N. coinciding with the drop in haemoglobin values.

N.P.N.—Both these fractions show a marked rise during the hyperthermic reactions. The N.P.N. rises from about 19 mgm. N per cent. to U.N. 51·72 mgm. N per cent. in the "laked" filtrate, returning to normal and increasing again to 25·54 mgm. N per cent. during the lesser reaction. In the "unlaked" filtrate the same phenomenon is observed, only on the lower level characteristic for this type of filtrate. The U.N. content runs parallel to the N.P.N., rising from 6·5 mgm. N per cent. to 37·10 mgm. N per cent ("unlaked" slightly lower), then returning to normal, followed by a slight increase to a little over the maximum range of the normal variation.

TABLE VIII.

S. 32113.												
DateTrime	28/9/31.	1/10/31.	21/10/31.	23/10/31.	27/10/31.	1/10/31. 21/10/31. 23/10/31. 27/10/31. 29/10/31.	3/11/31.	6/11/31.	6/11/31. 10/11/31. 12/11/31. 19/11/31.	12/11/31.	19/11/31.	1/12/31.
Temperature Reactions	N	N	E.	R	R	24	R	Z	24	R	점	Z
Haemoglobin gm. %	8.90	9.94	9.12	8.53	7.47	7.68	9.05	8.07	9.32	8.80	9.81	8.65
Sugar mgm. % L	66.92 58.80	53·20 43·48	38·00 35·71	60·61 54·65	62.89	53.48 40.82	71.43	48.58	57.47 52.36	58.14	42·01 42·37	38·46 31·75
T.N. gm. %	2.310	2.560	2.351	2.192	2.010	2.097	2.136	2.080	2.255	2.248	2.374	2.136
N.P.N. mgm. % I.	27.90	18.63 15.71	21.45 18.18	26.08 22.56	40.82 37.50	44.51 42.80	51.72 46.16	19.73	20.00 15.39	25·54 20·52	23.08 19.36	15.95 12.53
Coag. N gm. N % L	2.282	2.541	2.333	2.166	1.969	1.952	2.084	2.060	2.235 2.240	2.222	2.351	$\frac{2.120}{2.123}$
Urea mgm. N % L	13.80 28.98 13.80 28.98	6.50 13.65 6.40 13.44	10.40 28.84 10.95 23.10	15.00 31.50 14.33 30.03	26.97 56.70 26.03 54.60	33.78 70.98 34.10 71.61	37·10 77·70 35·04 73·50	9.31 19.53 8.95 18.90	7.08 14.91 7.36 15.54	12.87 27.09 12.56 26.46	10.94 22.89 10.77 22.68	4.80 10.08 4.13 8.61
Total Creatinine mgm. N % L	2·10 5·68 1·98 5·32	2·13 5·76 1·82 4·90	2.17 5.86 1.98 5.32	2.06 5.54 1.91 5.14	1.84 4.96 1.54 4.16	1.98 5.32 1.91 5.14	3.26 8.80 6.00	2.27 6.16 2.11 5.68	1111	3.80 10.28 2.74 7.40	2.66 7.20 2.42 6.54	2.31 6.26 2.06 5.54
Uric acid mgm. N % L		0.24 0.71 TL TL	0.23 0.69 0.08 0.23	0.22 0.66 TL TL	0.23 0.67 TL TL	0.27 0.80 TL TL	0.30 0.89 TL	0.17 0.50 TL	0.22 0.66 TL	0.29 0.87 TL	0.21 0.62 TL TL	0.23 0.70 0.15 0.44
Amino-acid mgm. N % L	5.30	6.28	5.60	5.60	5.00	3.89	6.51	5.83	5.60	7.18	6.67	6.36 5.18
R.N. mgm. N % U	6.70*	3.48	3.28 1.10	2.20	7.79	4.04	4.55	2.15	7.32† 3.79‡	1.46	2.58	$\frac{2.25}{1.00}$

- T.C.N.—In both filtrates there is a decrease, coinciding with the hyprethemia associated with the main heartwater reaction. Thereafter there is an increase reaching 3.60 mgm. N per cent. during the secondary reaction.
- U.A.N.—Shows a tendency towards a rise during main reaction.
- A.A.N.—Nothing unusual.
- R.N.—During the initial reaction this [fraction increased in amount to approximately 8 mgm. N per cent. and 4 mgm. N per cent. in "laked" and "unlaked" filtrates respectively.

CASE IX.

S. 29661: Died 6/11/31.

Temperature Chart IX.



History.—Sheep 29661, six-tooth hamel, carrying 3½ ins. wool 14/9/31, in good condition, weighing 77 fb. on 15/9/31 and 80 fb. on 6/10/31. Passed through bluetongue in July, 1931. Was placed on temperature 2/9/31 and was injected on 24/10/31 with 10 c.c. blood from sheep 31871 (vide) which was suffering from heartwater. The temperature reaction set in on the 7th day, the temperature steadily rising to reach 108° on the 4th day after the onset, remained at 108° for two days and then fell by crisis within 24 hours to 104°, the sheep dying on the 13th day p.i.

On post-mortem examination there was found to be a general cyanosis, slight hydrothorax and hydropericard, oedema of the lungs, fatty changes in the liver and the kidneys, tumor splenis, subendocardial and subepicardial haemorrhages, findings which support the diagnosis of heartwater.

TABLE IX.

S. 29661.						
Date	18/9/31.	30/9/31.	14/10/31.	27/10/31.	4/11/31.	6/11/31.
Temp. React	N	N	N	P.I.N.	R	R
<i>Hb.</i> gm. %	10.97	11 · 18	10.97	11.67	10.14	12-42
Sugar mgm. %L U	62·11 50·60	45·54 42·54	$50 \cdot 00 \\ 34 \cdot 40$	53·96 45·15	55·87 45·66	156·24 151·50
T.N. gm. %	2.370	2.480	2 • 465	2.458	2 · 262	2.532
<i>N.P.N.</i> mgm. % L U	22·22 18·52	30·28 24·00	18·79 14·77	20·00 16·27	40·54 36·48	$31 \cdot 74 \\ 25 \cdot 00$
Coag. N. L gm. N % U	$2 \cdot 348 \\ 2 \cdot 351$	2·450 2·456	2 · 446 2 · 456	2·438 2·442	$2 \cdot 221 \\ 2 \cdot 226$	2·500 2·505
Urea mgm. N % L	=	$14 \cdot 40$ $30 \cdot 24$ $14 \cdot 04$ $29 \cdot 40$	5·89 12·39 5·76 12·18	6.87 14.49 7.50 15.75	29·06 61·11 29·77 62·68	$ \begin{array}{r} 22 \cdot 00 \\ 46 \cdot 20 \\ 20 \cdot 05 \\ 42 \cdot 21 \end{array} $
Total Creatinine L mgm. N % U	$2 \cdot 13$ $5 \cdot 76$ $1 \cdot 67$ $4 \cdot 50$	$2 \cdot 31$ $6 \cdot 26$ $1 \cdot 49$ $4 \cdot 00$	$ \begin{array}{r} 2 \cdot 31 \\ 6 \cdot 26 \\ 1 \cdot 49 \\ 4 \cdot 00 \end{array} $	$2 \cdot 02$ $5 \cdot 42$ $1 \cdot 54$ $4 \cdot 16$	$2 \cdot 17$ $5 \cdot 86$ $1 \cdot 75$ $4 \cdot 70$	1·91 5·14 1·37 3·48
Uric Acid L mgm. N % U	0·25 0·76 —	0·22 0·67	0·24 0·71	0·21 0·62 —	0·26 0·77 —	0·25 0·76 0·18 0·53
Amino acid L mgm. N % U	6·73 5·96	9·72 ? 4·56	7·53 5·64	7·73 5·38	5·18 4·12	4·67 3·18
R.N. mgm. N % L U	14·11* 12·16†	4·63 3·91‡	2·39 1·88‡	3·17 1·85‡	3·87 0·84‡	$\begin{array}{c} 2 \cdot 91 \\ 1 \cdot 78 \end{array}$

* Includes "Urea Nitrogen.

† "Uric acid N" and "Urea N."

; "Uric acid N."

Salient Features of Analytical Data.

Sugar.—This shows a gradual increase up to 56 mgm. per cent. with a very high level (156 mgm. per cent.) a few hours before death on 6/11/31.

N.P.N.—Both evince the usual feature of an increase to 41 and 37 mgm. N per cent. respectively for N.P.N. in both filtrates succeeded by a decline on day of death. The U.N. curve is similar, being U.N.29 mgm. N per cent. (61 mgm. urea).

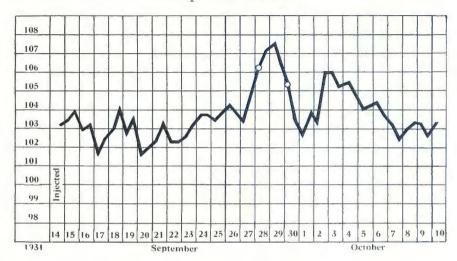
T.C.N.—Here a decline from 2.31 mgm. N per cent, to 1.91 mgm. N per cent. is noted.

A.A.N.—Shows a relatively low level a few hours before death.

CASES X, XI, AND XII.

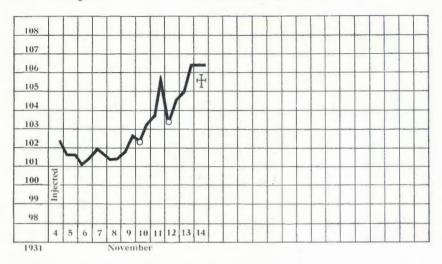
S. 31051: Recovered.

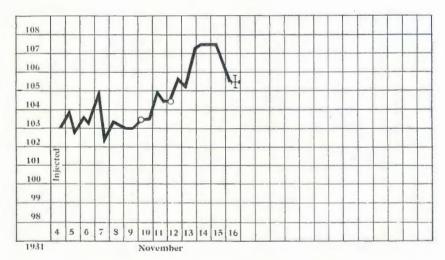
Temperature Chart X.



History.—Sheep 31051, four-tooth hamel, carrying 3 ins. wool, good condition, weighing 75 fb. on 15/9/31 and 74 fb. on 6/10/31. Passed through bluetongue in May, 1931. The animal was placed on temperature on 14/9/31 and injected on the same day with 20 c.c. of blood from a calf which had died of heartwater (natural infection). On the 13th day the temperature rose, reaching $107 \cdot 6^{\circ}$ on the 15th day, gradually subsiding to normal by the 24th day p.i.

Temperature Charts XI and XII. Sheep 31873 and 31144.





History.—Sheep 31873 and 31144. These sheep were injected with 10 c.c. blood each intrajugularly from S. 29661 (vide) on 4/11/31. Both temperature reactions set in on the 7th day, reaching $107 \cdot 4^{\circ}$ on the 10th day p.i., S. 31873 dying on the 11th day p.i. and S. 31144 on the 13th day at the height of the temperature reaction. Both were in good condition.

S. 31873 at the post-mortem examination (P.M. No. 10677 of 15/11/31) showed slight hydrothorax and hydropericard, subendocardial haemorrhages, hyperaemia and oedema of the lungs, ulcerative abomasitis and acute enteritis.

S. 31144 (P.M. No. 10678 of 16/11/31) showed anaemia, oedema of the lungs, hydrothorax, ascitis, subepicardial and subendocardial haemorrhages, nodular enteritis, tumor splenis and fatty changes in the liver and kidneys.

TABLE X.

S. 31051.				
Date		3/9/31.	28/9/31.	30/9/31.
Temperature Reaction		N	R	R
Haemoglobin gm. %		9.12	8.90	9.13
Sugar mgm. %	£ U		60·00 37·00	$46 \cdot 51 \\ 40 \cdot 00$
Total Nitrogen gm. N %	L	2 · 350	2 · 300	2 · 297
Non-Protein Nitrogen mgm. %	L U	18·70 13·00	$24 \cdot 50 \\ 22 \cdot 30$	$\begin{array}{c} 27 \cdot 03 \\ 20 \cdot 98 \end{array}$
Couquable Nitrogen gm. N %	L U	$2 \cdot 331 \\ 2 \cdot 338$	$2 \cdot 275 \\ 2 \cdot 278$	$2 \cdot 270 \\ 2 \cdot 276$
Urea mgm. N %	L U		$ \begin{array}{r} 13 \cdot 37 \\ 28 \cdot 14 \\ 13 \cdot 04 \\ 27 \cdot 30 \end{array} $	$12 \cdot 77$ $26 \cdot 88$ $12 \cdot 99$ $27 \cdot 30$
Total Creatinine mgm. N %	L U		$2 \cdot 23$ $6 \cdot 00$ $1 \cdot 75$ $4 \cdot 70$	$1 \cdot 91$ $5 \cdot 14$ $1 \cdot 78$ $4 \cdot 80$
Uric Acid mgm. N %	L U	0·21 0·63 TL TL	TL TL TL TL	TL TL TL TL
Amino Acid mgm. N %	L U	$5 \cdot 74 \\ 4 \cdot 67$	5·49 5·11	5·11 3·99
Rest Nitrogen mgm. N %	L U		3·41* 2·40*	7 · 24* 2 · 22*

^{*} Includes " Uric Acid N."

TABLE XI.

TABLE XII.

S. 31873.			S. 31144.	
DateTime	10/11/31.	12/11/31.	10/11/31.	12/11/31
Temperature Reactions	R	R	R	R
Haemoglobin gm. %	13.41	7_	12.98	10.14
Sugar mgm. % L	42·37 33·56	46·95 38·46	39·37 38·46	51·02 42·37
T.N. gm. N %	2.710	2.570	2.696	2.528
N.P.N. mgm. % L	19·08 14·63	30·00 25·64	$16.94 \\ 12.25$	23·06 18·04
Coag. N. gm. N % L	2·691 2·695	$2 \cdot 540 \\ 2 \cdot 546$	$2.679 \\ 2.684$	$2.505 \\ 2.510$
Urea mgm. N % L U	7·00 14·70 6·02 12·60	$ \begin{array}{r} 17 \cdot 00 \\ 35 \cdot 70 \\ 15 \cdot 00 \\ 31 \cdot 50 \end{array} $	4·28 9·03 4·25 9·03	$9 \cdot 10$ $19 \cdot 11$ $9 \cdot 02$ $18 \cdot 90$
Total Creatinine mgm. N % L U		2·66 7·20 2·54 6·86		2·73 7·58 2·48 6·70
Uric Acid mgm. N % L	0·22 0·66 TL TL	0·30 0·91 TL TL	0·22 0·66 TL TL	0·27 0·86 TL TL
Amino acid mgm. N % L	$6 \cdot 36 \\ 5 \cdot 49$	6·93 4·91	6·51 4·67	$7 \cdot 57 \\ 5 \cdot 22$
R.N. mgm. N % L	5·48* 3·12†	3·11 3·19‡	5·93* 3·33†	3·39 2·32‡

^{*} Includes "Total Creatinine N."

Salient Features of Analytical Data.

These three cases are grouped together owing to the small number of analyses available for each.

- *Hb.*—In case of XII a decrease from 12·98 gm. per cent. to 10·42 gm. per cent. occurs.
- N.P.N.—All show slight increases, the relatively low levels when compared and with other cases being probably due to the acute nature of U.N. the reactions.
- T.C.N.—In Case X a decrease is noted; in the other cases no comparative figures are available.

^{† &}quot; "Total Creatinine N " and "Uric Acid N."

^{‡ &}quot; "Urie Aeid N."

GENERAL SUMMARY AND DISCUSSION.

Haemoglobin (Hb.).—The haemoglobin content during any particular period normally shows variations not only in the same animal from practically day to day, but also in different animals of the same species. Factors such as age, sex, condition, time of watering in relation to the period at which the blood is examined, all contribute towards a variable Hb. content. It follows, therefore, that a "mean" Hb. curve can only be arrived at if a number of successive determinations are undertaken. In such a case, although there may be variations, the "mean" level can readily be noted. In the present series of investigations care was taken to obviate the introduction of an anaemia due to too frequent bleedings of one and the same animal.

Under pathological conditions various circumstances may produce a change in Hb. content, generally in the direction of oligo-chromaemia, hyperchromaemia being less frequently encountered.

With regard to heartwater no very striking changes in the Hb. content as a sequel to infection emerge. An analysis of the cases presented show that (a) a tendency towards oligo-chromaemia is present in over 50 per cent. of cases (Cases I, III, IV, V, VI, VIII and XII). In some cases the decrease is direct, e.g. in Case I from 13.79 gm. per cent. to 11.67 gm. per cent.; in Case VIII from 9·13 gm. per cent. to 7·68 gm. per cent.; in others an initial drop is succeeded by an increase usually towards the termination of the temperature reaction or shortly before death. This decrease is not associated with a destruction of erythrocytes by the virus or its metabolic products, such as is encountered in redwater (piroplasmosis) or anaplasmosis. In my opinion physical factors play the important rôle in producing these alterations. The increased water intake associated with hyperrhexia, tending to increased water ingestion, would tend towards the creation of hydraemia or dilution of the blood, leading to a decreased Hb. content per unit of blood, although the total Hb. would remain the same. On the other hand, the transudation of plasma into the peritoneal, pleural and pericardial cavities and the interstitial tissues of the lungs, especially if occurring rapidly at any particular stage of the disease, would tend towards a condition of a concentration of the blood. This transudation is probably a fairly rapid process; sheep killed in the earlier stages of the disease rarely show well-marked accumulations of transudate. Furthermore, the course of the disease is frequently so rapid that animals often survive only a short time after the onset of the temperature reaction. Where they survive one would expect an equally rapid return to the "mean" Hb. level by the absorption of the transudate, or of water into the blood, the osmotic forces tending to establish the status quo.

- (b) In some cases there is no definite change in the Hb. in either direction (Cases II and VII). If the above conception is correct one could assume in explanation that either the analyses were not performed at the psychological moment as far as the onset of the transudation is concerned, or that the absorption of water into the blood occurred as rapidly as the loss of plasma through transudation.
- (c) In Cases V and IX no distinct variations occurred beyond a rise in Hb. shortly before death.

Sugar (S).—The sugar content of the blood in heartwater shows a distinct increase in all cases studied, e.g., Case I, from 61–93 mgm. per cent, Case IX 56–156 mgm. per cent. In the majority of cases there is a decrease towards the end of the temperature reaction or just shortly before death in fatal cases.

This applies in both "laked" amd "unlaked" filtrates. Case VI provides an interesting exception—in the "laked" the increase is from \pm 40 mgm. to 96 mgm. per cent., but in the "unlaked" only from \pm 33–44 mgm. per cent., so that the "laked" contained over twice as much blood sugar at the peak point than the "unlaked." The decrease in several cases (III and VIII) continues to below the initial "mean" level. The increase is not in all cases absolute, i.e., does not necessarily proceed beyond the high side of normal, but the increase is evidenced by the gradual and definite rise from a lower level to a higher one. As previously stated the normal range lies between 45–55 mgm. per cent. although figures lower and above these limits are encountered, though relatively rarely. These figures agree closely with those of Völker (1929). In a case in which the "mean" lies at \pm 35 mgm. per cent. an increase to 55 mgm. per cent. should in my opinion be looked upon as abnormal, provided of course that the 35 mgm. per cent. reflects the true "mean" for that animal under its present environment and on the given ration.

Total Nitrogen (T.N.).—The T.N. in about half the cases (I, III, IV, V, VI, VII, VIII) shows a decrease; in the remaining cases there are minor variations but not in any specific direction. A partial decrease in the T.N. is a priori to be expected owing to the transudation of plasma into the body cavities and lungs. This drain of nitrogenous material is, however, rapidly replaced, as can be noted in cases ending in recovery, where shortly after the return of temperature to normal the T.N. has reached its previous level. In Case I there is a decrease from $2 \cdot 774$ to $2 \cdot 294$ gm. N per cent., which is followed by a rise to $2 \cdot 611$ gm. N on the day prior to death.

Non-Protein Nitrogen (N.P.N.).—In the majority of cases an increase in the N.P.N. in both filtrates has been noted. The increases vary from about 15–36 mgm. N per cent. (Case II), 15–26·55 mgm. N per cent. (Case II), 20–30 mgm. N per cent. (Case III), 20–64 mgm. N per cent. (Case IV), 16–40 mgm. N per cent. (Case VII), 19–52 mgm. N per cent. (Case VIII) and 20–40 mgm. N per cent. (Case IX). Cases V and VI are interesting in that the "normal" level before injection of virus was on the high side and no distinct increases are noted. This N.P.N. retention follows the temperature reaction, returning to normal practically with cessation of the hyperthermic state. This is particularly well illustrated in Case VIII, where a severe and long drawn out temperature reaction is followed by a brief return to normal, whereafter a second less severe reaction sets in. The N.P.N. increases during the first reaction from 19–52 mgm. N per cent., drops to 20 mgm. N per cent. with drop of temperature to normal, and rises again to 25·50 mgm. N per cent. during the second reaction.

The U.N. is primarily responsible for this increase, the other constituent N fractions either remaining normal (A.A.N.) or showing a tendency to decrease. The total content and variations of the U.A.N. are too small appreciably to influence the N.P.N. in either direction. That, however, the U.N. is not solely responsible become evident by the observation that usually during the period of highest N.P.N. concentration, the R.N. (i.e., rest-nitrogen or undetermined nitrogen) represents in a number of cases from 5–9 mgm. N per cent. instead of the normal 2–4 mgm. N per cent. for "laked" and less for "unlaked" filtrates.

Urea Nitrogen (U.N.).—The U.N. has been found to be greatly increased in heartwater, e.g., Case II 4–22 mgm. N per cent., Case IV 7–32 mgm. N per cent., Case VII 4–24 mgm. N per cent., Case VIII 6·5–37 mgm. N per cent., Case IX 6–29 mgm. N per cent., the increase and decrease (with recovery

cases) running parallel with the temperature reaction; the "mean" level being rapidly attained at the end of the hyperthermia. A high U.N. figure is generally associated with renal impairment or dysfunction. In heartwater there is in most cases found a fatty degeneration of the renal cortex, but I am inclined to consider the cardiac disturbance, as evinced by the cyanosis of mucous membranes, as the chief factor in the severe U.N. retention. According to this conception the cardiac condition leading to circulatory disturbances and thereby to renal dysfunction is of more significance in U.A. retention than the degeneration of the kidney. In two out of the three cases of recovery the U.N. remained at below 18 and 13 mgm. N per cent, respectively, in the third case the U.N. reached the high figure of 37 mgm. N per cent., and, as already mentioned, the recovery here was a matter of doubt for several days. It could theoretically be advanced that the virus plays some rôle in the production of the urca by toxicogenic protein decomposition, but in view of the rapid excretion of urea via the kidneys, this would have to be extensive before an accumulation in the blood could be expected. In such a case one would expect a more marked reduction in the protein nitrogen than is actually the case.

Uric Acid Nitrogen (U.A.N.).—No common factor can be traced through the data collected, there being a tendency towards an increase in some cases, the reverse in others, and in others again no change in either direction.

"Total creatinine" Nitrogen (T.C.N.).—The values found for T.C.N. are somewhat irregular and permit of no definite conclusions. On the whole there exists a tendency towards a decrease during heartwater, e.g., Case I 2·6-I·91 mgm. N per cent., Case IX 2·31-I·91 mgm. N per cent., Case X 2·23-I·19 mgm. N per cent., in "laked" and corresponding decreases in "unlaked" filtrates. In most cases where no decrease during the temperature reaction has occurred there is a slight increase again shortly before death. For this latter observation a possible explanation lies in the contracted agonal period, where the animal when down performs galloping movements with the limbs and exhibits severe tetanic spasms of the musculature resembling somewhat the syndrome encountered in cases of strychnine poisoning. It is generally accepted that creatinine is formed in the musculature and that its excretion is in some measure an expression of muscular activity. Separate determinations for creatine and creatinine were not done, and it is, therefore, not possible to state whether the creatine or the creatinine fraction, or both, are responsible for the decreases and increases found.

Amino-acid Nitrogen (A.A.N.).—No distinctive variations noticeable; in two cases a slight decrease prior to death is noted (Cases III and IX).

Conclusion.

In heartwater increases in the concentrations of sugar, N.P.N. and U.N. in both filtrates are recorded. The T.C.N. shows a tendency towards decreasing, with a rise in some cases just before death. The Hb. content varies, but is generally in the direction of a decrease.

B.—BLUETONGUE IN SHEEP (CATARRHAL FEVER).

The condition referred to here is the catarrhal fever or "Bluetongue" found in South Africa.

It has been defined as an inocuable disease of sheep principally affecting the mucous membranes of the mouth, nose, and intestines, very often accompanied with inflammation of the laminae of the feet. It is caused by an ultra-visible virus, probably transmitted from sheep to sheep by some flying insect. Animals which have recovered are immune and no longer carry the virus in the blood.

For more detailed information the articles listed in the bibliography at the end of this article may be consulted, the symptomatology being only very briefly described here.

Clinically abortive, acute and subacute forms are distinguished.

In the abortive form no symptoms, beyond a temperature reaction, can be seen.

Acute or subacute form.—With artificial infection the incubation period is generally 2–4 days, the hyperthermia lasting 5–7 days. Other symptoms are usually observed 7–10 days p.i.—generally salivation, swelling and catarrh of the buccal, mucous and nasal membranes and gums; occasionally also of the conjunctiva. Small sores may be noted on the lips. If more severe, an haemorrhagic inflammation may develop, the tongue becoming very swellen and brownish-red in colour, usually associated with a shiny greyish nasal discharge. In many cases a laminitis is observed. The animals lose in condition and may shed their wool (rare). The mortality varies—it is generally low but may be as high as 50 per cent.

(a) SELECTION AND TREATMENT OF THE SHEEP.

In the present researches nine sheep were utilised. All were artificially infected and all reacted. Blood was examined before infection and thereafter, particularly during the temperature reaction. The sheep used were all bastard Merinos, purchased in bluetongue-free areas and kept stabled. The ration given was the same as for the heartwater sheep (vide). In order to reduce wireworm infection to a minimum, the sheep received regular anthelminthic treatment with the Government Wireworm Remedy (monthly).

The strain of virus employed was a "field" strain, subinoculated into S. 34498 which succumbed to the infection, and although marked temperature reactions were obtained in the sheep, none died. Under the favourable experimental conditions, with its regular feeding, protection from the inclemencies of the weather, plentiful supply of water, etc., bluetongue is rarely fatal.

(b) CHEMICAL METHODS OF ANALYSES AND TECHNIQUE.

These are the same as those referred to under "Heartwater." In the arrangement and discussion the same general plan as for the previous disease has been adhered to.

(c) EXPERIMENTAL DATA.

(1) Normal Range of Constituents.

For the compilation of the following only data obtained from the sheep used for the bluetongue experiments prior to infection have been utilised. These data are additional to those tabulated under "Heartwater" and, although they agree with them closely, they are in general on a somewhat lower level. The former data were gathered during September to November, 1931, the latter during June to August, 1932, and the existing differences represent seasonal variations, there being a tendency for a progressive decrease in the concentrations of the various constituents during the winter. The present summary only represents twelve analyses of sheep blood of various ages, and for data comprising a much larger number of analyses of the normal blood of sheep under the same conditions I would refer to the work done by Hamersma (1933).

Haemoglobin.

Minimum-maximum variation: 9.1-16.5 gm. per cent. Average: 13.0 gm. Hb. per cent.

Sugar.

Minimum-maximum variation "laked": 37-55.5 mgm. per cent. Average "unlaked": 47.36 mgm. per cent.

Minimum-maximum variations "unlaked": 31.3-50.5 mgm. per cent. Average "unlaked": 39.1 mgm. per cent.

Percentage differences between "laked" and "unlaked" 10.3-27.6 per cent. Average percentage difference: 17.4 per cent.

Total Nitrogen.

Minimum-maximum variation: 2.4-3.3 gm. N per cent. Average: 2.8 gm. N per cent.

Non-Protein Nitrogen.

Minimum-maximum variations "laked": 13.6-20 mgm. N per cent. Average "laked": 16.5 mgm. N per cent.

Minimum-maximum variations "unlaked": 9.4-15.3 mgm. N per cent. Average "unlaked": 12.5 mgm. N per cent.

Percentage differences between "laked" and "unlaked" 9.7–33.2 per cent.

Average percentage difference: 25 per cent.

Urea Nitrogen.

Minimum-maximum variation "laked": 3.1-7.3 mgm. N per cent. Average "laked": 4.9 mgm. N per cent.

Minimum-maximum variation "unlaked" 3·0-7·3 mgm. N per cent. Average "unlaked": 4·7 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 4.1 per cent.

Total Creatinine Nitrogen.

Minimum–maximum variations "laked": $1\cdot67-2\cdot35$ mgm. N per cent. Average "laked": $2\cdot08$ mgm. N per cent.

Minimum-maximum variations "unlaked": 1.43-2.00 mgm. N per cent.

Average "unlaked": 1.72 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 17.3 per cent.

Uric Acid Nitrogen.

Minimum-maximum variations "laked": 0·15-0·26 mgm. N per cent. Average "laked": 0·22 mgm. N per cent.

Minimum-maximum variations "unlaked": 0·10-0·22 mgm. N per cent.

Average "unlaked": 0·13 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 41 per cent.

Amino-acid Nitrogen.

Minimum-maximum variations "laked": 4·1-6·1 mgm. N per cent. Average "laked": 5·30 mgm. N per cent.

Minimum-maximum variations "unlaked": 2·72-3·90 mgm. N per cent. Average "unlaked": 3·20 mgm. N per cent.

Percentage difference between "laked" and "unlaked" average 40 per cent.

Rest Nitrogen.

Minimum-maximum variations "laked": 2.65-6.73 mgm. N per cent. Average "laked": 4.13 mgm. N per cent.

Minimum-maximum variations "unlaked": 1.54-3.58 mgm. N per cent.

Average "unlaked": 2.33 mgm. N per cent.

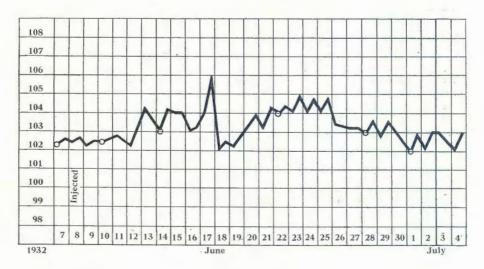
Percentage difference between "laked" and "unlaked" average 43.6 per cent.

(2) BLUETONGUE DATA.

CASE I.

Sheep 25142.

Temperature Chart I.



History.—A six-tooth ewe, carrying about 2 ins. wool, in good condition. Placed on temperature 17/5/32, and injected subcutaneously with 2 c.c. blood on 8/6/32. The reaction was severe but not in as much as the hyperthermia was concerned but rather in its effect on the animal, which showed cyanosis of the buccal and nasal mucous membranes and laminitis. Furthermore, as a result of this infection it shed its wool completely and lost condition. Discharged 27/8/32.

Table 1.—S. 25142. Bluelongue.

Date. Time	7/6/32.	10/6/32.	14/6/32.	22/6/32.	28/6/32.	1/7/32.	5/7/32.	14/7/32.	20/7/32.
Temperature Reaction	N	N	P.I.N.	P.I.N.	R	3	Z	×	N
Haemoglobin gm. %	15.98	14.95	16.54	11.39	8.49	8.49	9.32	9.81	9.94
Sugar mgm. % L	44.64	11		117.64	63 · 69 44 · 05	59.52 53.76	34·60 29·94	65.79 59.88	80.65 68.03
N.P.N. mgm. % L	20.63 13.63	16.30 10.82	22.39 15.82	66 · 60 53 · 55	26.31 23.44	18.75	21.43 18.36	11.32 8.33	13.82 12.00
Total N. gm. %	3.074	3.00	3.032	2.416	2.220	2.322	2.360	2.318	2.290
Coag. N gm. N % U	3.053	2.984 2.989	3.009	2.350	2.194 2.197	2.313 2.317	2.339	2.307	2.276 2.278
Urea mgm. N % L , U % L mgm. N % U % U , U % U , U % U , U %	6.69 14.07 6.97 14.49	3.83 7.98 3.69 7.77	9.87 20.79 9.42 19.74	49.00 102.90 43.44 91.14	15.53 32.55 15.39 32.34	7.33 15.33 7.26 15.33	11.27 23.73 10.56 22.26	2.51 2.25 2.33 4.83	6.41 13.44 6.12 12.81
Total Creatinine mgm. N %	2.27 6.16 1.82 4.90			4.02 10.80 2.76 7.44	2.57 6.96 2.01 5.40	2.27 6.16 1.49 4.00	1.87 5.02 1.44 3.86	1.87 5.02 1.54 4.16	1.78 4.80 1.54 4.16
Uric aeid mgm. N % L. ", UA % U. mgm. N % U	0.25 0.76 0.12 0.35	1111		0.22 0.67 0.11 0.32	0.25 0.76 0.11 0.33	1 1	0.23 0.69 0.18 0.55	$\begin{array}{c} 0.21 \\ 0.64 \\ 0.18 \\ 0.53 \end{array}$	0·19 0·58 0·14 0·41
Amino acid mgm. N % U	5.69	5.56	5.56	5.60	5.18	5.83	4.32	4.24	4.24 2.50
Rest N. mgm. N %	6.73	6.91*	8.96*	6.76 3.57	2.78	3.32+	3.74	2.49	1.20
Plasma	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.
			1 to 1 to 1	N. W.	A Tanaha	A Tandard on 17 Make	77 N. 17		

* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."

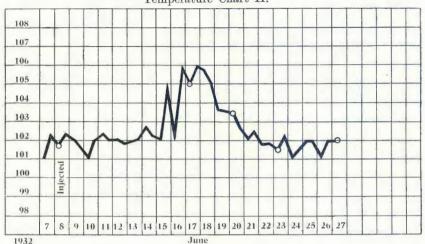
† Includes "Uric Acid. N."

Main Features of Analytical Data.

Hb.—A drop from 15·73-8·49 gm. % is noted, the decrease 504 cidding with the onset of the fever reaction. The return to its previous IIb. level was rather gradual, \$\text{Sugar_8}\$ only being reached after about four weeks.

\$\text{Sugar_8}\$ Sugar_9. Suga

Case II. Sheep 22204. Temperature Chart II.



History.—A four-tooth ewe, carrying 2 ins. wool, in good condition. Placed on temperature 17/5/32 and injected subcutaneously with 2 c.c. virulent blood on 8/6/32. After an incubation period of six days the temperature rose. Towards the end of the reaction the animal appeared rather dull and listless and showed slight cyanosis of the buccal mucous membrane. It made an uneventful recovery. Discharged 15/7/32.

Case III. S. 34896.

History.—A two tooth hamel, 3 ins. wool, in good condition. Placed on temperature 18/5/32 and injected subcutaneously with 2 c.c. blood on 8/7/32. Incubation period five days. The animal showed an injection of the buccal mucous membranes and slight laminitis, but recovered very quickly from these symptoms. Discharged 27/8/32.

Bluetongue. Table 2.—S. 22204.

Date	19/5/32.	8/6/32.	17/6/32.	20/6/32. 10.30 a.m.	23/6/32.	27/6/32.	30/6/32;	5/7/32.	14/7/32.	23/7/32. 7.15 a.m.
Temperature Reaction	Z	Z	R	A.	Z	Z	N	Z	N	N
Haemoglobin gm. %	16.50	14.49	15.53	18.20	14.28	13.31	12.92	13.48	12.79	12.79
Sugar mgm. % L	42.92	44.35 39.84	50.00		53.48	46.08	45.05 40.32	49.26 40.82	43.10	49.75
Total N. gm. %	3.256	2.906	3.092	3.144	2.843	2.640	2.689	2.696	2.590	2.759
N.P.N. mgm. % L	15.46	13.63	18·40 16·10	20.00	25.86 21.43	15.00 12.50	14.71	15·41 11·25	13.04	15.00
Coag. N gm. N % L	3.241	2.892	3.074	3.124	2.816	2.625	2.674	2.681	2.557	2.744
Urea mgm. N % L Mgm. N % U U U U U U	4.13 8.61 4.00 8.40	3·13 6·51 3·00 6·30	8.25 17.43 8.00 16.80	12.60 26.46 11.99 25.20	13.29 27.93 12.69 26.67	5.76 12.18 5.49 11.55	4.71 9.87 4.65 9.87	4.26 9.03 4.14 8.61	3.88 8.19 3.55 7.56	3.85 8.19 4.00 8.40
Total Creatinine mgm. N % L mgm. TC % U , TC % U	1.82 4.90 1.49 4.00	2·17 5·84 2·01 5·40	2·23 6·00 1·91 5·14		1.61	2·11 5·68 1·86 5·62	2·23 6·00 1·64 4·40	2·10 5·68 1·44 3·86	2·27 6·16 2·17 5·84	2·10 5·68 1·92 4·90
Uric acid mgm. N % L L mgm. N % U	0.23 0.69 0.18 0.53	0.15 0.45 0.09 0.27	0·14 0·41 0·10 0·29		0·13 0·40 0·07 0·20	0.15 0.44 0.09 0.28	0.27	0.20 0.61 0.19 0.57	0.17 0.50 0.13 0.40	0.11 0.33 0.07 0.20
Amino acid mgm. N % L	6.09	4.11	4.64	4·46 2·70	5.30	4.83	4.38	4.64	4.36	3.89
Rest N. mgm. N % L	3.19	4.07	3.14	2.94*	7.14†	2.15	3.12	4.12	2.36	5.05
Plasma	m.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.
* Includes "Total Creatinine" and "Uric Acid Nitrogen,"	ine " and " U	frie Acid Nitz	ogen."	† Includes	† Includes "Total Creatinine."	tinine."	‡ Include	† Includes "Uric Acid."	d."	

† Includes "Total Creatinine." Main Features of Analytical Data. "Includes "Total Creatinine" and "Uric Acid Nitrogen."

Hb.—Gradually decreased from 15·53–12·79 gm. %, the decrease coinciding with the return of the temperature to normal.

N.P.N.—Shows an increase from approximately 15-26 % and from 12-21 mgm. N % for "laked" and "unlaked" filtrates, respectively. The return to normal cocurred a few days after the cessation of the febrile reaction.

U.N.—An increase from 3-13 mgm. N % is noted.

U.A.N.—A distinct tendency towards a decreased level during the febrile stage in both filtrates occurred.

Sugar, T.C.N., A.A.N., R.N.—Nothing unusual.

Table 3.—S. 34896. Bluetongue.

Date		30/6/32.	12/7/32.	15/7/32.	18/7/32.	21/7/32.	28/7/32.	5/8/32.
Temperature Reactions	tions	Z	P.I.N.	R	R	R	R	N
Haemoglobin gm.	%	14.08	14.28	14.08	13.50	16.50	12.94	14.28
Sugar mgm. %	HD	46.08	43.29	71.43	51-02	54·35 48·08	44.05	46.51
T.N. gm. %		2.864	2.710	2.724	2.605	2.655	2.696	2.717
N.P.N. mgm. %	I	18.90	23.69	21.43 14.28	18.52	18.63	17.02 12.00	16.31 11.53
C.N. gm. N %	%	2.845	2.686	2.703 2.710	2.586	2.636	2.679	2.701
Urea	mgm. N % L " U % U mgm. N % U	7.33 15.33 7.33 15.33	10.11 21.21 9.87 20.79	8.39 17.64 8.09 17.01	7.16 15.12 7.06 14.91	6.40 13.44 6.27 13.23	5.25 11.13 5.19 10.92	4.40 9.24 4.13 8.61
T.C.	mgm. N % L mgm. N % U	2.23 6.00 1.51 4.08	2.35 6.36 1.78 4.80	2.42 6.54 1.82 4.90	2·17 5·84 1·78 4·80	2.10 5.68 1.93 5.14	2.23 6.00 1.75 4.70	2.17 5.84 1.82 4.90
Uric acid	mgm. N % L " UA % U mgm. N % U		0.27 0.80 0.11 0.32	0.25 0.74 0.12 0.35	0.22 0.66 0.11 0.33	0.23 0.68 0.13 0.40	$\begin{array}{c} 0.17 \\ 0.52 \\ 0.07 \\ 0.22 \end{array}$	0.23 0.69 0.11 0.32
Amino acid mgm. N %.	N %	3.33	6.36	5.76	4.67	3.04	6·09 4·00	6.09
R.N. mgm. N %	T	3.86*	4.60	4.61	4.30	5.23	3.28	3.42
Dlasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

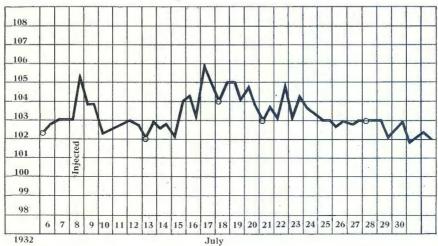
* Includes " Uric Acid N."

Main Features of Analytical Data.

Sugar., N.P.N., and U.N.—Slight tendency towards a higher level which, however, is not above the upper limits of normal. A.A.N.—A drop during the hyperthermia is noted. Hb., T.N., U.A.N., T.C.N., and R.N.—Nothing unusual.

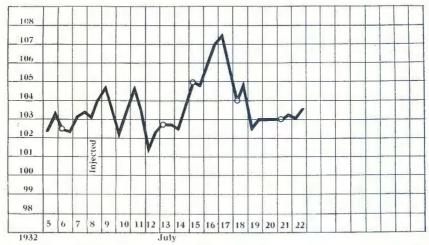
Case IV. S. 34898.

Temperature Chart IV.



History.—A four tooth hamel, $3\frac{1}{2}$ ins. wool, in good condition. Placed on temperature 19/5/32 and injected subcutaneously with 2 c.c. blood on 8/7/32. After an incubation period of seven days an irregular mild fever reaction supervened. From the 23rd-26th a slight laminitis was noted. It made an uneventful recovery. Discharged 27/8/32.

CASE V. S. 34885. Temperature Chart V.



History.—A four-tooth hamel, $3\frac{1}{2}$ ins. wool, in good condition. Drafted into experiment 18/5/32 and injected subcutaneously with 2 c.c. blood on 8/7/32. Incubation period five days, and beyond a hyperthermia no other symptoms were shown. Discharged on 27/8/32.

Table 4. S. 34898. Bluetongue.

Date Time	6/7/32.	13/7/32.	18/7/32.	21/7/32.	28/7/32.	5/8/32.
Temp. R	N	P.I.N.	R	R	N	N
Hb. gm. %	12.59	11.24	11.67	12.28	11.14	11.14
Sugar mgm. % L	41·49 32·26	48·08 37·45	58·14 47·17	48·31 38·02	$42 \cdot 92 \\ 35 \cdot 21$	47·85 39·68
Total N. gm. %	2.598	2.430	2 · 332	2.360	2.444	2.479
N.P.N. mgm. % L U	17·65 14·54	15·79 10·71	16·30 11·07	13·63 10·79	13·65 10·71	$19 \cdot 23 \\ 15 \cdot 00$
Coag. N. L gm. N % U	$2.580 \\ 2.583$	2·414 2·419	2·316 2·321	$2 \cdot 346 \\ 2 \cdot 349$	2·430 2·433	2·460 2·464
Urea mgm, N % L ,, U % mgm. N % U ,, U %	$7 \cdot 20$ $15 \cdot 12$ $7 \cdot 03$ $14 \cdot 70$	4·10 8·61 3·94 8·19	5·45 11·55 5·45 11·55	4·48 9·45 4·56 9·66	3·88 8·19 3·40 7·14	7.51 15.75 7.33 15.33
T.C. mgm. N % L ,, TC % mgm. N % U ,, TC %	1·98 5·32 1·67 4·50	$2 \cdot 01$ $5 \cdot 40$ $1 \cdot 71$ $4 \cdot 60$	1·49 4·00 1·82 4·90	$2 \cdot 01$ $5 \cdot 40$ $1 \cdot 98$ $4 \cdot 32$	2·50 6·74 2·35 6·36	$2 \cdot 35$ $6 \cdot 36$ $2 \cdot 04$ $5 \cdot 50$
Uric acid mgm. N % L ,, UA % mgm. N % U ,, UA %	$0.25 \ 0.76 \ 0.12 \ 0.36$	0·19 0·56 0·08 0·25	$0 \cdot 21 \\ 0 \cdot 64 \\ 0 \cdot 12 \\ 0 \cdot 36$	$0.19 \\ 0.57 \\ 0.14 \\ 0.41$	$0.13 \\ 0.40 \\ 0.08 \\ 0.24$	0·20 0·60 0·11 0·33
Amino acid L mgm. N % U	5·56 3·77	5·60 3·67	4·00 2·52	4·12 2·92	$5 \cdot 18 \\ 3 \cdot 72$	5·64 3·89
Rest N. L mgm. N % U	2·65 1·95	3·89 1·31	5·15 1·18	2·83 1·79	2·96 1·16	3·53 1·63
Plasma	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Except for a tendency towards a slight decrease in the A.A.N. level during the febrile reaction, no other blood changes occurred.

Table 5.
S. 34885. Bluetongue.

Date	6/7/32.	13/7/32.	15/7/32.	18/7/32.	21/7/32.	28/7/32
Temp. R	N	P.I.N.	R	R	N	N
Hb. gm. %	10.35	9.81	9.94	11.14	8.88	9.40
Sugar mgm. %. L U	$48 \cdot 31 \\ 40 \cdot 16$	44·84 33·78	62·50 49·02	$60 \cdot 24 \\ 49 \cdot 75$	51·28 46·95	$52 \cdot 63$ $45 \cdot 25$
Total N. gm. %	2.542	2 · 402	2 · 290	2.304	2.059	2 · 220
N.P.N. mgm. % L U	$17 \cdot 04 \\ 12 \cdot 94$	$17.65 \\ 13.57$	17·85 13·04	$\begin{array}{c} 23 \cdot 20 \\ 16 \cdot 76 \end{array}$	20·00 16·48	15·00 11·11
Coag. N. L gm. N % U	$2 \cdot 525 \\ 2 \cdot 529$	$2 \cdot 384 \\ 2 \cdot 388$	$2 \cdot 272 \\ 2 \cdot 277$	$2 \cdot 281 \\ 2 \cdot 287$	$2 \cdot 039 \\ 2 \cdot 039$	$2 \cdot 205$ $2 \cdot 205$
Urea mgm. N % L ,, U % mgm. N % U ,, U %	$4 \cdot 61$ $9 \cdot 66$ $4 \cdot 26$ $9 \cdot 03$	$5 \cdot 37$ $11 \cdot 34$ $5 \cdot 06$ $10 \cdot 71$	$6 \cdot 46$ $13 \cdot 65$ $6 \cdot 69$ $14 \cdot 07$	11.50 24.15 11.43 23.94	$\begin{array}{r} 10 \cdot 43 \\ 21 \cdot 84 \\ 10 \cdot 03 \\ 21 \cdot 00 \end{array}$	$4 \cdot 33$ $9 \cdot 03$ $4 \cdot 19$ $8 \cdot 82$
Total Creatin. mgm. N % L " TC % mgm. N % U " TC %	$2 \cdot 17$ $5 \cdot 84$ $1 \cdot 91$ $5 \cdot 14$	$2 \cdot 01$ $5 \cdot 70$ $1 \cdot 78$ $4 \cdot 80$	2·17 5·84 1·91 5·14	$ \begin{array}{c} 2 \cdot 57 \\ 6 \cdot 96 \\ 1 \cdot 82 \\ 4 \cdot 90 \end{array} $	$ \begin{array}{c c} 1 \cdot 91 \\ 5 \cdot 14 \\ 1 \cdot 86 \\ 5 \cdot 02 \end{array} $	$2 \cdot 50 \\ 6 \cdot 74 \\ 2 \cdot 10 \\ 5 \cdot 64$
Uric acid mgm. N % L " UA % mgm. N % U ", UA %	$0 \cdot 26 \\ 0 \cdot 70 \\ 0 \cdot 12 \\ 0 \cdot 36$	0.20 0.60 0.10 0.30	$0.22 \\ 0.65 \\ 0.12 \\ 0.36$	0.23 0.69 0.11 0.34	$0 \cdot 21$ $0 \cdot 64$ $0 \cdot 12$ $0 \cdot 36$	0·15 0·46 0·08 0·25
Amino acid L mgm, N % U	5·60 4·11	6·03 3·91	$5 \cdot 38 \\ 3 \cdot 59$	$\begin{array}{c} 3 \cdot 84 \\ 2 \cdot 32 \end{array}$	3·89 2·69	5-60 3-65
Rest N. L mgm. N % U	$4 \cdot 40 \\ 2 \cdot 54$	$\frac{4 \cdot 04}{2 \cdot 72}$	$3.62 \\ 0.75$	$\begin{array}{c} 5 \cdot 06 \\ 1 \cdot 08 \end{array}$	$\begin{array}{c} 3 \cdot 56 \\ 1 \cdot 70 \end{array}$	$\begin{array}{c} 2 \cdot 42 \\ 1 \cdot 09 \end{array}$
Plasma	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

Sugar.—Shows a slight rise.

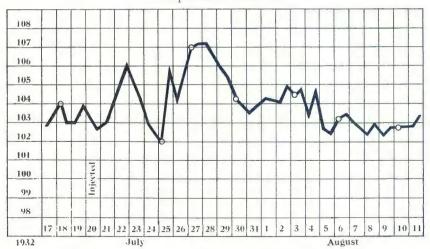
N.P.N.—Shows a slight increase from approximately 17–23 mgm, N % and 13–17 mgm, N %.

U.N. —An increase from 4.5–11 mgm. % is observed.

A.A.N.—Slight decrease during the temperature reaction.

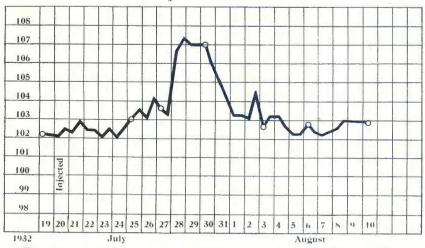
Hb., T.C.N., U.A.N., R.N., T.N.—Nothing unusual.

Case VI. S. 34872. Temperature Chart VI.



History.—A two-tooth hamel, $3\frac{1}{2}$ ins. wool, in good condition. Injected subcutaneously 2 c.c. blood on 27/7/32, and after an incubation period of two days a severe and prolonged hyperthermia supervened. Towards the end of the reaction the animal became dull and went down, but showed no other symptoms. Discharged 27/8/32.

CASE VII. S. 34870. Temperature Chart VII.



History.—A two-tooth hamel, 3 ins. wool, good condition. Placed on emperature 18/5/32 and injected subcutaneously with 2 c.c. blood on 20/7/32. ncubation period four days. Except for a mild laminitis and for a slight eddening of the buccal mucous membrane, recovery was uneventful.

Table 6.—S. 34872. Bluetongue.

Date	8/7/32.	18/7/32.	25/7/32.	27/7/32.	30/7/32.	3/8/32.	6/8/32. 7.15 a.m.	10/8/32.
Temperature Reaction	Z	Z	R	R	R	R	N	N
Haemoglobin gm. %	14.08	13.87	11.53	11.92	11.94	10.27	11.53	10:87
Sugar mgm. % U	41.49	37·04 31·25	49.26	48.31	75.19	71.43	46.51	47.40
Total N. gm. %	2.752	2.780	2.444	2.472	2.434	2.318	2.325	2.400
N.P.N. mgm. % U	15.00	15.00	13.04	15.87	17.97	20·00 15·79	17.65	15.40
Coag. N. gm. N % L	2.737	2.765	2.431	2.456	2.416	2.298	2.307	2.385
Urea mgm. N % L " " " " " " " " " " " " " " " " "	5.45 11.55 5.15	4.19 8.82 4.13	3.55 3.55	6.40 13.44 5.97	9.52 19.95 9.25	9.00 18.90 9.00	7.00 14.70 6.84	5.25
%	10.92	8.61	7.56	12.60	19.53	18.90	14.20	1
Total Creatinine mgm. N % L TC % D mgm. N % U , U , TC %	1.67 4.50 1.43 3.86	1.91 5.14 1.61 4.32	2·23 6·00 1·91 5·14	2.04 5.50 1.67 4.50	2·10 5·68 1·78 4·80	2.27 6.16 1.98 5.32	2·01 5·40 1·61 4·32	2.27
Uric acid mem. N % L	0.50	0.19	0.22	0.19	0.15	0.23	0.50	0.15
mgm. N %	$0.60 \\ 0.12 \\ 0.36$	0.56 0.11 0.34	$0.67 \\ 0.16 \\ 0.47$	$0.57 \\ 0.11 \\ 0.33$	0.10 0.29	$0.68 \\ 0.15 \\ 0.46$	0.60 0.14 0.41	0.48
Amino acid mgm. N % L	4.24 2.92	4.97	4.38	3.84	4.52	5.00	5.56	5.18
Rest N. mgm. N % U	3.44	3.74	2.21	2.40	1.68	3.50	2.88	2.55
Plasma	n.u.	n.u.						

Main Features of Analytical Data.

Sugar.—Increases to 75 mgm. % during the latter part of the febrile reaction. N.P.N., U.N.—Show relatively slight, but distinct, increase during reaction. T.C.N., U.A.N., A.A.N., T.N., R.N.—Nothing unusual.

Table 7.—S. 34870. Bluetongue.

Date		. 19/7/32.	25/7/32.	27/7/32.	30/7/32.	3/8/32.	6/8/32.	10/8/32.
Temperature Reaction	n	Z	P.I.N.	P.I.N.	M	R	N	Z
Haemoglobin gm. %	0/	. 11.39	13.31	13.12	12.79	13.31	11.39	11.14
Sugar mgm. %	T C	51.28	55·55 41·15	59.17	62.50	55.55	57.80	40.00
Total N gm. %		2.437	2.738	2.696	2.514	2.402	2.435	2.394
N.P.N. mgm. %	T C	18.18	16.66	14.85 11.03	23.08	18.75	17.14	18.75
Coag. N. gm. N %	T C	2.419	2.721	2.681	2.491	2.383	2.418	2.375
Urea	mgm. N %	5·25 10·93 5·35 11·34	4.88 10.29 3.69 7.77	4.48 9.45 4.40 9.24	10.43 21.84 10.17 21.42	6.55 13.86 6.27 13.23	4.26 9.03 4.00 8.40	6.00
T.O.	mgm. N %. TC %. mgm. N %. TC %.	2.23 6.00 2.01 5.40	2.50 6.74 1.75 4.70	2·23 6·00 1·71 4·60	2.27 6.16 2.04 5.50	1.82 4.90 1.49 4.00	1.75 4.70 1.49 4.00	1.87 5.14 —
Uric acid	mgm. N % L " UA %. mgm. N % U ", UA % U	0.22 0.67 0.12 0.36	0.26 0.78 0.19 0.50	0.21 0.64 0.11 0.33	0.20 0.61 0.08 0.24	0.27 0.80 0.16 0.47	0.27 0.82 0.13 0.40	0.17
Amino acid mgm. N %	n% N	5.38	5.18 3.33	6.38	5.38	5.60	5.83	5.51
R.N. mgm. N %	n n	5.10	3.84	2.75	4.80	4.51	5.03	5.20
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.

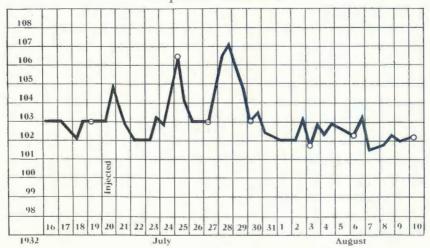
Sugar.—Slight tendency to rise.

N.P.N. and U.N.—Both show a distinct increase, e.g., N.P.N. from 16-23 and from 11-16 mgm. N %, and U.N. from 4-10·4 mgm. N %, with a rapid return to normal after the temperature reaction has ended.

Hb., T.C.N., U.N.N., A.A.N., R.N., and T.N.—Nothing unusual.

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CASE VIII. S. 34900. Temperature Chart VIII.



History.—A four-tooth hamel, 3 ins. wool, good condition. Was placed on temperature 18/5/32 and injected subcutaneously on 20/7/32 with 2 c.c. blood. After an incubation period of four days a temperature reaction set in, but apart from this and a slight dullness, the animal showed no other symptoms. Discharged 27/8/32.

CASE IX. S. 34912. Temperature Chart IX.



History.—A two-tooth hamel, $3\frac{1}{2}$ ins. wool, in good condition. Drafted into experiment 19/5/32 and injected subcutaneously with 2 c.c. blood on 20/7/32. After an incubation period of five days a severe hyperthermia set in, reaching 108° in 72 hours, but, except for a slight dullness and loss of appetite, no other symptoms were noted. Discharged 27/8/32.

Table 8.—S. 34900. Bluetongue.

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Date. Time.		19/7/32.	25/7/32.	27/7/32.	30'7'32.	3 '8/32.	6/8/32. 7.15 a.m.	10/8/32.
Temperature Reactions	:	×	R	R	*	×	×	Z
Haemoglobin gm. %	:	11 -24	10.76	11.14	12.13	10.99	11.96	11.24
Sugar mgm. %	122	56·18 50·00	64 · 94 55 · 50	60 · 61 49 · 50	66 ·67 54 ·05	59 ·52 51 ·55	58 · 14 49 · 50	45.60
T.N. gm. %	:	2.451	2 -374	2 .493	2.535	5.409	2.640	2.520
N.P.N. mgm. %	T L	16.48	17.35	16.66	22 · 90 16 · 04	19 · 73 15 · 79	17.65	15.80
Coug. N. gm. N %	75	2 · 435 2 · 440	2.357	2·476 2·482	2.512 2.519	2 :389	2 -622 2 -628	2.504
Urea mgm. N %	L U	3 :27 6 :93 3 :00 6 :30	5 · 45 7 · 35 5 · 35 11 · 34	4 · 40 9 · 24 4 · 13 8 · 67	9.42 19.74 9.00 18.90	7.00 14.70 6.67 14.07	4.71 9.87 4.88 10.29	8.67
Total Creatinine mgm. N % % % % % % % % % % % % % % % % % %	I D	2.35 6.36 1.82 4.90	2 · 43 6 · 54 2 · 35 6 · 36	2 · 23 6 · 00 1 · 67 4 · 50	2.23 6.00 1.67 4.50	2 · 10 5 · 68 1 · 82 4 · 90	2.10 5.68 1.71 4.60	5 · 84
Uric acid mgm, N %. UA %. mgm, N %. mgm, N %. UA %.	T L	0.22 0.67 0.15 0.45	0.26 0.78 0.20 0.59	0 · 22 0 · 67 0 · 13 0 · 39	0.20 0.59 0.11 0.32	0.25 0.74 0.15 0.45	0.29 0.86 0.14 0.41	0.20
Amino acid mgm. N %	הנד	5 · 79 3 · 50	4 · 83	5·26 3·41	5.98	5.96	6.22	5.15
<i>R.N.</i> тgm. N °°.	Jh	4.85 2.81	4.39	4 · 55 1 · 52	5·07 1·45	4.42 2.91	4.33	4 · 15
Plasma		n.u.	n.u.	n.u .	n.u.	n.u.	n.u.	n.u.

Sugar, X.P.X. and U.X.—These constituents have a tendency to rise slightly during the febrile reaction, reaching concentration just over the upper level of the normal range.

Hb., T.C.X., U.A.X., A.A.X., R.X. and T.X.—Nothing usual. Main Features of Analytical Data.

S. 34912. Bluetongue. TABLE 9.

DateTrime.		19/7/32.	25/7/32.	27/7/32.	30/7/32.	3/8/32.	6/8/32. 7.15 a.m.	10/8/32.
Temperature Reactions	ions.	Z	P.I.N.	R	24	R	Z	×
Haemoglobin gm. %	%	9.13	9.32	88.88	10.35	9.50	. 9.32	9.42
Sugar mgm. %	H C	56.82	52.91 47.52	62 · 50 56 · 50	58.82 48.54	75 · 19 68 · 03	63 · 69 57 · 14	52.25
T.N. gm. %		2.195	2.206	2.150	2.304	2.164	2.024	2.052
N.P.N. mgm. %	n	14 .42	14.42 12.50	14.28 10.71	21.42	20.54 15.31	17 · 11 13 · 16	15.65
Coag. N. gm. N %	n	2.181	2.192 2.194	2.136	2.283	2.143	2.007	2.036
Urea	mgm. N % L mgm. U % U ,, U % U	3.35 7.14 2.98 6.30	3.81 4.98 7.98	4.26 9.03 9.03 8.19	10.77 22.68 10.56 22.26	8.75 18.48 8.30 17.43	4·43 9·24 4·13 8·61	4.26 9.03 —
Total Creatinine	mgm. N % L " TC % U mgm. N % U	2.17 5.84 2.01 5.40	2.35 6.36 2.04 5.50	2·04 5·50 1·75 4·70	2.66 7.20 2.27 6.16	2·10 5·68 1·82 4·90	2.10 5.68 1.82 4.90	2.04
Uric acid	mgm. N % L " UA % U mgm. N % U	$\begin{array}{c} 0.19 \\ 0.56 \\ 0.22 \\ 0.67 \end{array}$	0.25 0.74 0.26 0.62	0.21 0.62 0.13 0.40	0.17 0.52 0.07 0.22	0.24 0.73 0.16 0.48	0.22 0.65 0.16 0.48	0.14
Amino acid mgm. N	N %	5.51 4.24	5·00 3·84	4.67	5.22 3.15	5.38 3.50	6.67	5.79
R.N. mgm. N %	n n	3.20	3.01 2.82	3.10	2.60	4.07	3.69 2.53	3.42
Plasma		0.u.	n.u.	n.u.	n.u.	n.u.	n.u	n.u.

Sugar.—Increases from about 52-75 mgm. % and from 50-68 mgm. % for "laked" and "unlaked" filtrate, respectively. N.P.N.—Increases from 14-21 and from 13-15 at the acme of the reaction. U.N.—Increases from 3-10-5 mgm. N %. Hb., U.A.N., T.C.N., A.A.N., R.N., and T.N.—Nothing unusual.

SUMMARY OF ANALYTICAL DATA FROM THE PATHOLOGICAL ASPECT.

In the nine cases of bluetongue examined, Case I represented the severest reaction, the other eight less severe, and Case IV the least, although the strain used must be considered as virulent. It was obtained from Natal from an outbreak of bluetongue in which mortalities were reported and was responsible for several deaths when subinoculated into sheep here at Onderstepoort. The conditions under which the experimental animals were kept, i.e., regular feeding, watering and shelter, are probably responsible for the absence of more severe reactions and mortalities. The severe temperature reactions noted indicate in some measure the virulency of the strain.

If the analytical data are reviewed as a whole, the following emerges:-

Haemoglobin.

In only two cases (I and II) can any definite change be established, e.g., in Case I the Hb. drops from 15·7–8·5 gm. per cent., with a relatively slow recovery, and in Case II from 15·5–12·9 gm. per cent., the decrease in both cases coinciding with the hyperthermic reaction. It should be noted that these two cases represent the more severe reactions. In the other seven cases no distinct changes in either direction are noticeable.

Sugar.

An increase is noted in all except two cases (II and IV), viz., Case I from 45–117.6 mgm. per cent.; Case III, 45–71 mgm. per cent.; Case V, 45–60 mgm. per cent.; Case VI up to 75 mgm. per cent., etc., in "laked" and correspondingly in "unlaked" filtrates. The increase may be very striking, as in Case I, or merely an increase to just over the upper limits of normal, depending on the severity of the reaction.

Non-Protein Nitrogen.

Shows an increase in all cases except Case IV, varying from \pm 18-66 mgm. N per cent. in Case I, from 15–26 mgm. N per cent. in Case II, from 17–23 mgm. N per cent. in Case V, from 16–23 mgm. N per cent. in Case VII, and from 14–21 mgm. N per cent. in Case IX, with smaller increases in the other cases. The concentration in the "unlaked" filtrates rises correspondingly.

Urea Nitrogen.

Shows increase in all cases except Case IV, e.g., Case I from 5-49 mgm. N per cent., Case II 3.5-13 mgm. N per cent., Case V 4.5-11 mgm. N per cent., Case VII 4-10.4 mgm. N per cent., and Case IX from 3-10.5 mgm. N per cent. The increase in U.N. is solely responsible for the rise in the N.P.N. fraction recorded above.

Total Creatinine Nitrogen.

Except for a slight rise recorded in the severe reaction in Case I, no variations outside the normal range are noticeable.

Total Nitrogen.

This remains unchanged except in Cases I and II, i.e., cases associated with a decrease in the Hb. level of the blood.

Amino-Acid Nitrogen, Uric Acid Nitrogen and Rest Nitrogen. No appreciable alterations occur.

CONCLUSION.

In bluetongue of sheep there is generally an increase in the sugar, N.P.N. and U.N. fraction of the blood. In severe cases a decrease in Hb. and a corresponding decrease in T.N. is recorded. The degree of the variations correspond approximately to the severity of the reaction.

III.—GENERAL SUMMARY.

- (1) The total nitrogen and haemoglobin of whole blood, and the sugar, urea, amino-acid, "total" creatinine, uric acid and non-protein nitrogen of "laked" and "unlaked" blood filtrates of sheep have been determined.
- (2) Determinations were made during the pre-infection period ("normal"), the incubation period, the actual hyperthermic reaction stage, and in recovery cases during the convalescent stage, until the level of the blood constituents had returned to normal.
- (3) "Normal" data in respect of the above constituents for both "laked" and "unlaked" filtrates have been separately detailed.
- (4) As far as differences in the two types of filtrates are concerned, the concentrations in "laked" filtrates are in all cases higher than in the "unlaked," the difference being least in the case of urea.
- (5) Temperature charts to show the nature and type of the reaction, as well as the periods at which blood was withdrawn, have been incorporated.
- (6) Well marked changes are recorded in Heartwater and Bluetongue, the degree of the change corresponding approximately to the severity of individual reactions.

ACKNOWLEDGMENTS.

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REFERENCES.

In addition to the publications referred to in the text a few additional references referring more particularly to the clinical and pathological aspect have been included, since these aspects were only very briefly dealt with.

In spite of a very extensive search no references bearing directly on the conditions investigated here could be found.

HEARTWATER.

- ALEXANDER, R.A. (1931). Heartwater. The present state of our knowledge of the disease. 17th Rept. of Dir. of Vety. Serv. and Anim. Indust., Union of S.A., Part I, p. 98 (extensive bibliography).
- COWDRY, E. V. (1926). Cytological Studies on Heartwater: I and II. 11th and 12th Rept. Dir. of Vety. Educ. and Res., p. 161.
- FOURIE, P. J. J. (1931). The haematology and pathology of haemonchosis in sheep. 17th Rept. of Dir. Vety. Serv. and Anim. Indust., Union of S.A., p. 495.

- GRAF, H. (1933). Chemical Blood Studies: I. Comparative Studies on Blood, "Laked" and "Unlaked" blood filtrates of animals in health and disease, with particular reference to methods and technique employed. This Journal.
- HAMERSMA, P. J. (1933). Chemical Blood Studies: VI. A serial study (over a 12-month period) of some organic constituents in "laked" and "unlaked" blood filtrates of healthy sheep of various ages. Next number of this Journal.
- JACKSON, C. (1931). The microscopic diagnosis of heartwater. 17th Rept. Dir. Vety. Serv. and Anim. Indus., Union of S.A., p. 161.
- JACKSON, C., AND NEITZ, W. O. (1932). On the actiology of Heartwater. 18th Rept. of Dir. Vety. Serv. and Anim. Indust., Union of S.A., Part I, p. 49.
- KNUTH AND DU TOIT (1921). Handbuch der Tropenkrankheiten, Vol. VI (extensive bibliography).
- THEILER, A. (1903-4). Heartwater. Annual Rept. Govt. Vety. Bact., 1903-4, p. 114.
- VÖLKER, R. (1929). Blutzuckeruntersuchungen an gesunden und kranken Tieren I. Arch. Wiss. Prakt. Tierheilkunde, Vol. 59, p. 16.

BLUETONGUE.

- DU TOIT, P. J. (1929). Nature and duration of immunity against Bluetongue in sheep. 15th Rept. Dir. Vety. Serv., Union of S.A., p. 69.
- DU TOIT, P. J. (1929). Studies on the virus of Bluetongue. Idem., p. 79.
- KNUTH and DU TOIT (1921). Handbuch der Tropenkrankheiten, Vol. VI. (extensive bibliography).
- THEILER, A. (1904). Bluetongue in Sheep. Annual Rept. Govt. Bact., 1904-5, p. 110.

Chemical Blood Studies .- This Journal.

- GRAF, H.: I. Comparative studies on blood, "laked" and "unlaked" blood filtrates of animals in health and disease, with particular reference to mtehods and technique employed.
- JORDEN, T. J., AND GRAF, H.: II. A contribution to the determination of urea in animal blood filtrates ("laked" and "unlaked").
- GRAF, H.: III. Comparative studies on "laked" and "unlaked" blood filtrates of sheep in health and during Heartwater and Bluetongue.
- GRAF, H.: IV. Comparative studies on "laked" and "unlaked" blood filtrates of horses in health and during Horsesickness.
- GRAF, H.: V.—Comparative studies on "laked" and "unlaked" blood filtrates of cattle in health and during Anaplasmosis and Piroplasmosis.